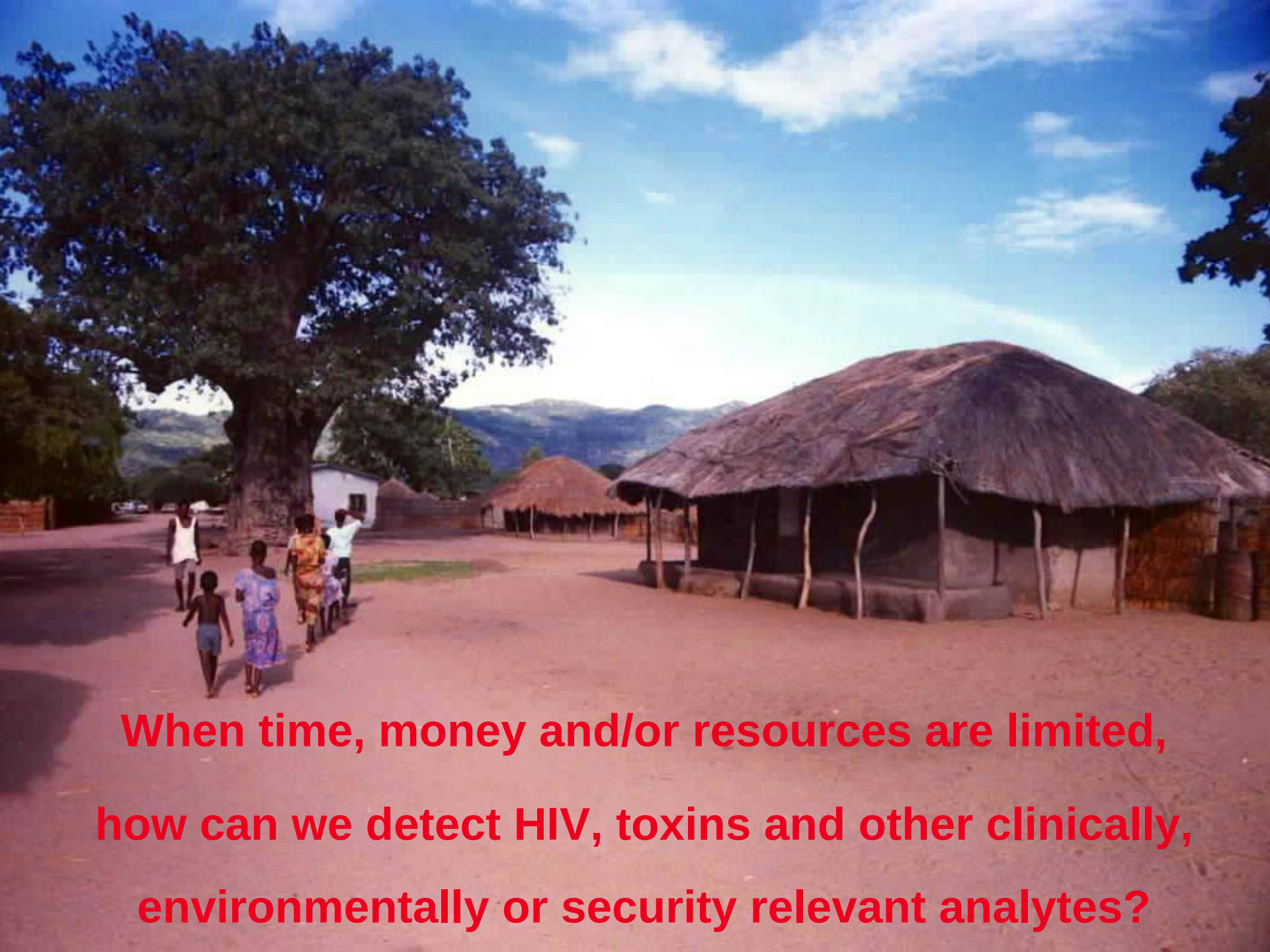
Better Living

Through Biosensors

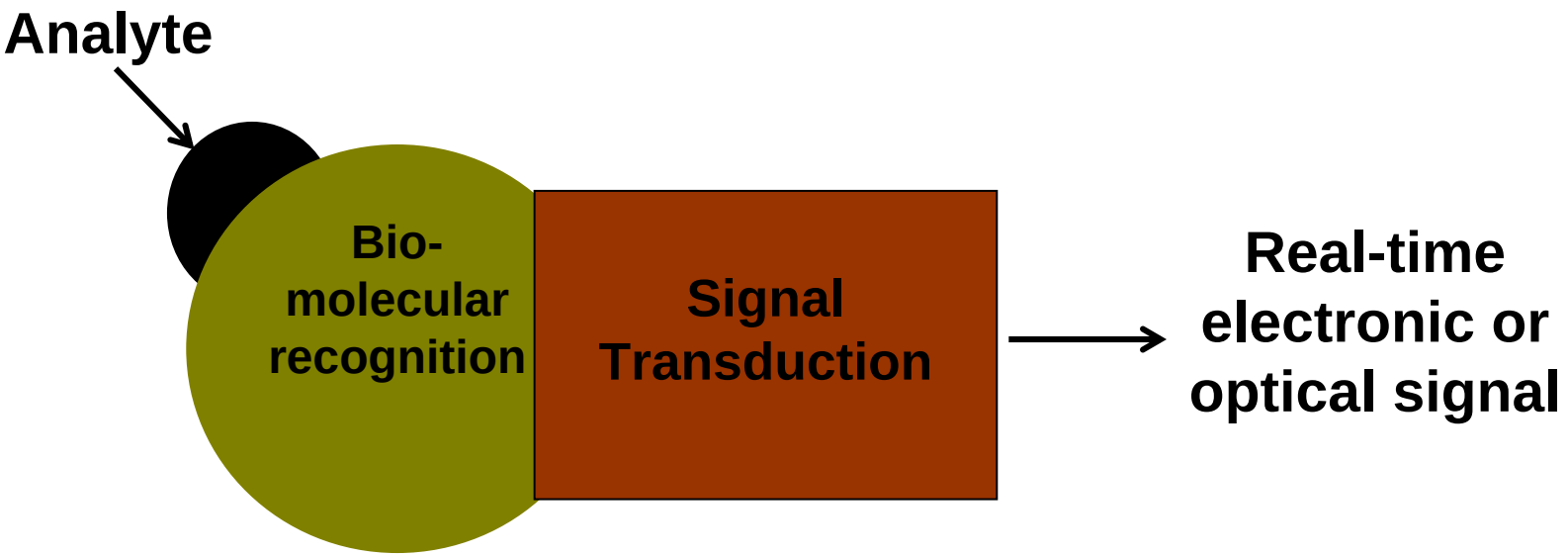


When time, money and/or resources are limited,

how can we detect HIV, toxins and other clinically,

environmentally or security relevant analytes?

What is a Biosensor?

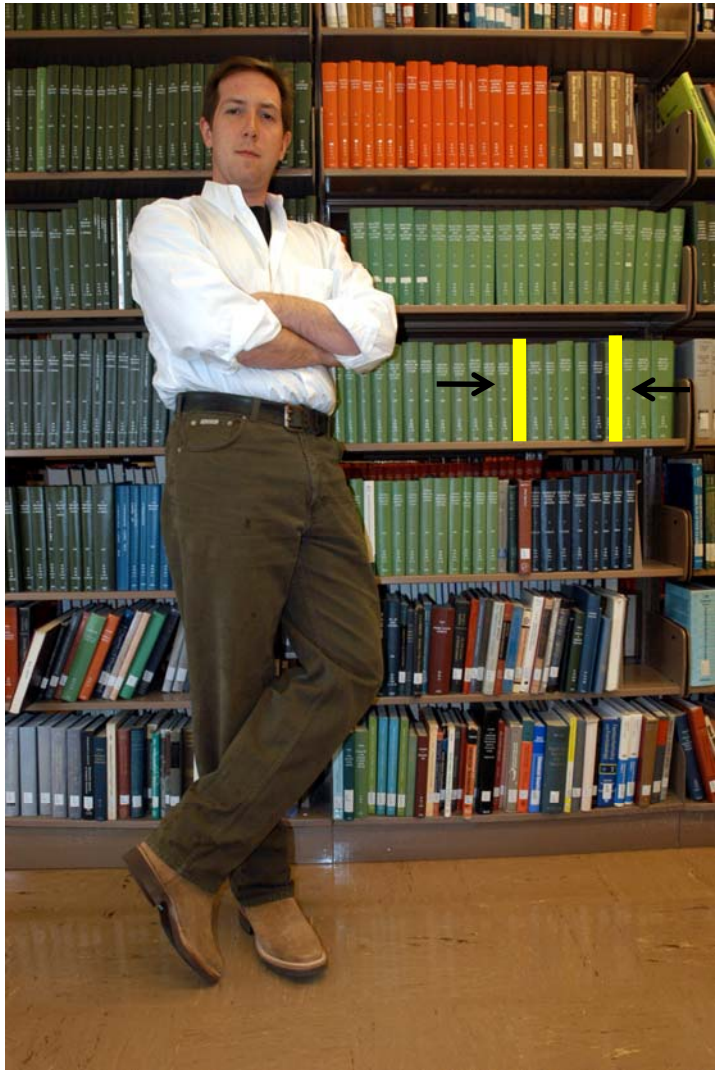


A sensor that exploits the specificity of biological recognition, such as performed by enzymes, antibodies, nucleic acids or cells.

Why Biology-based Sensors?

- **General: virtually any water-soluble target**
- **Sensitive: affinities approaching 10^{-18} M**
- **Specific: single residue/base discrimination**

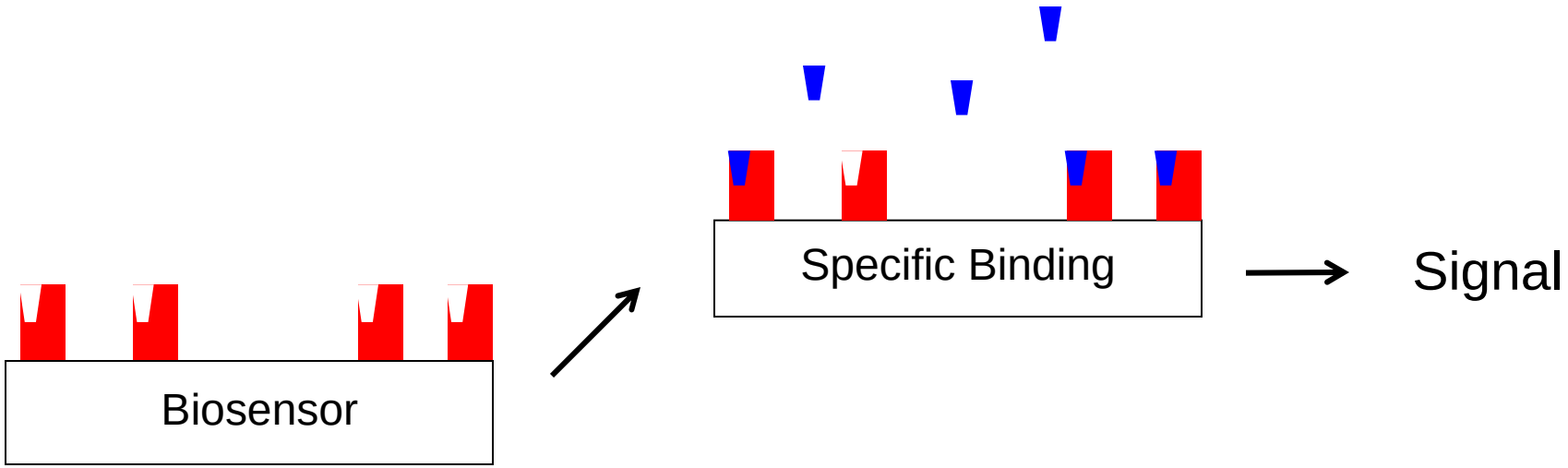
The “Biosensor Gap”



>100 papers/year are published with key words “Biosensor” and “Rapid”

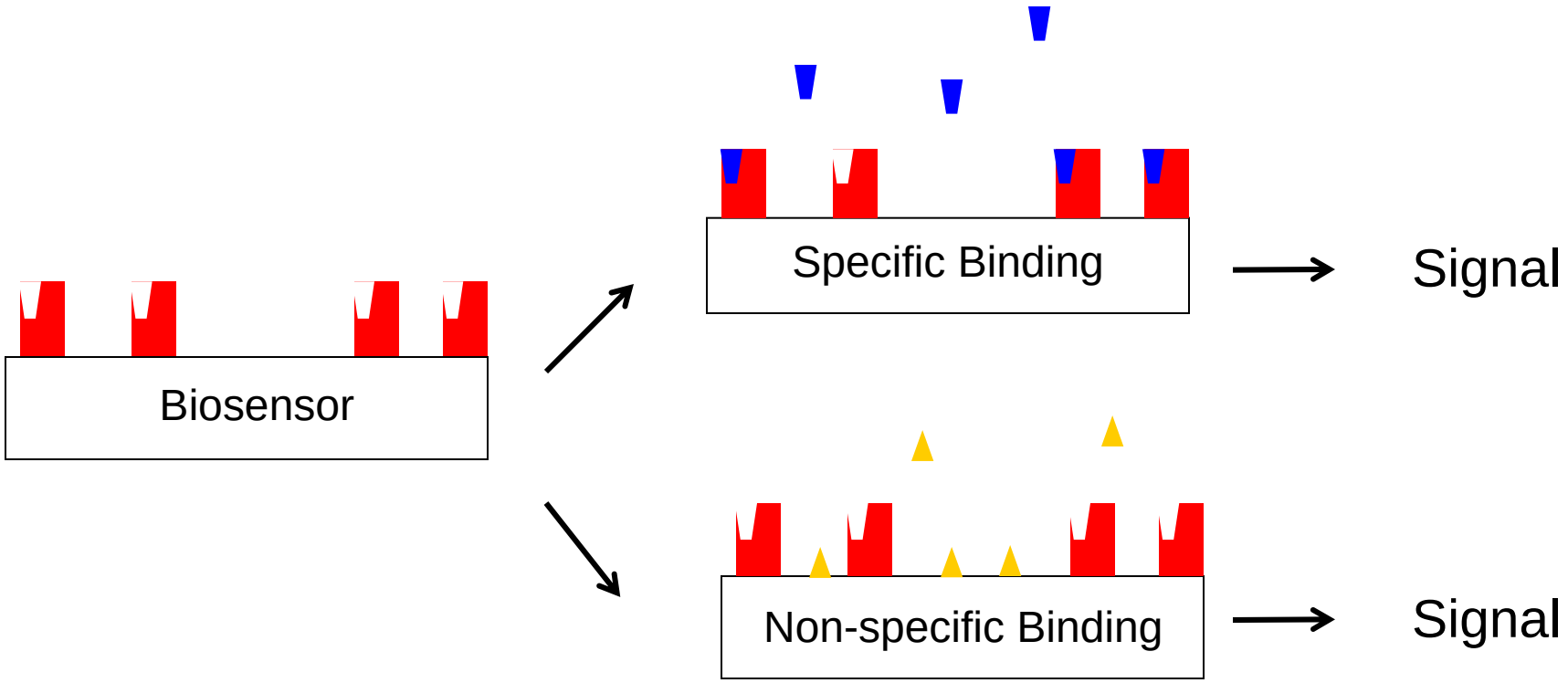
Yet there is only a single real-time biosensor on the market today

“Traditional” Biosensor



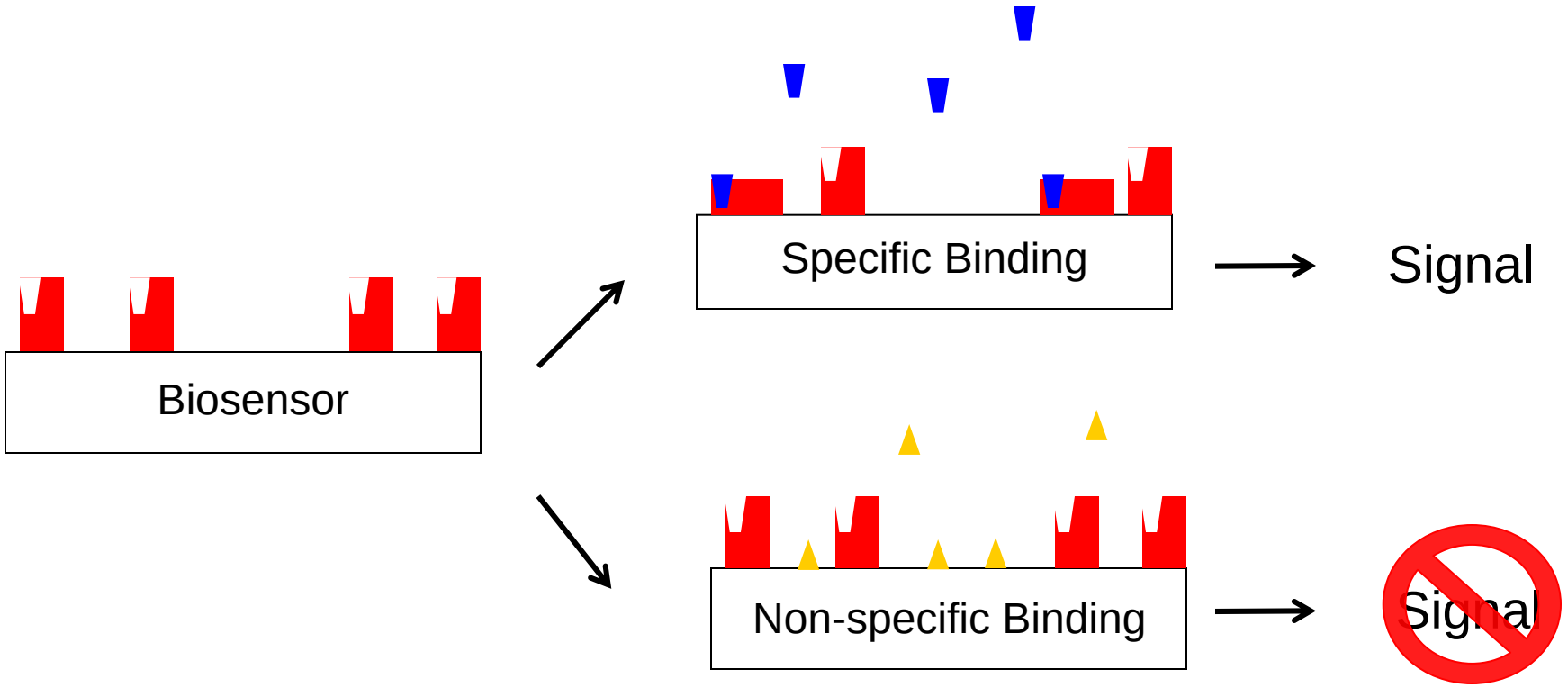
Measure changes in adsorbed mass, polarizability, sterics or charge

Achilles' Heel



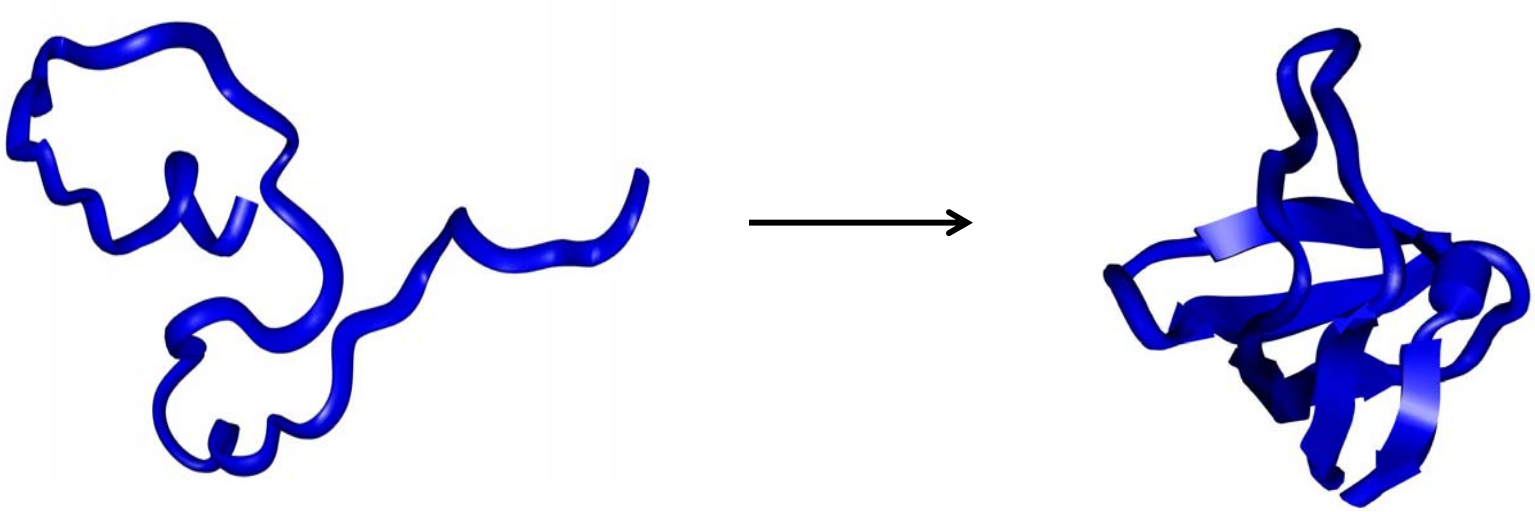
A critical, and to date unavoidable, failure mode of traditional biosensor is signals arising from the non-specific binding of contaminants.

Signal Transduction

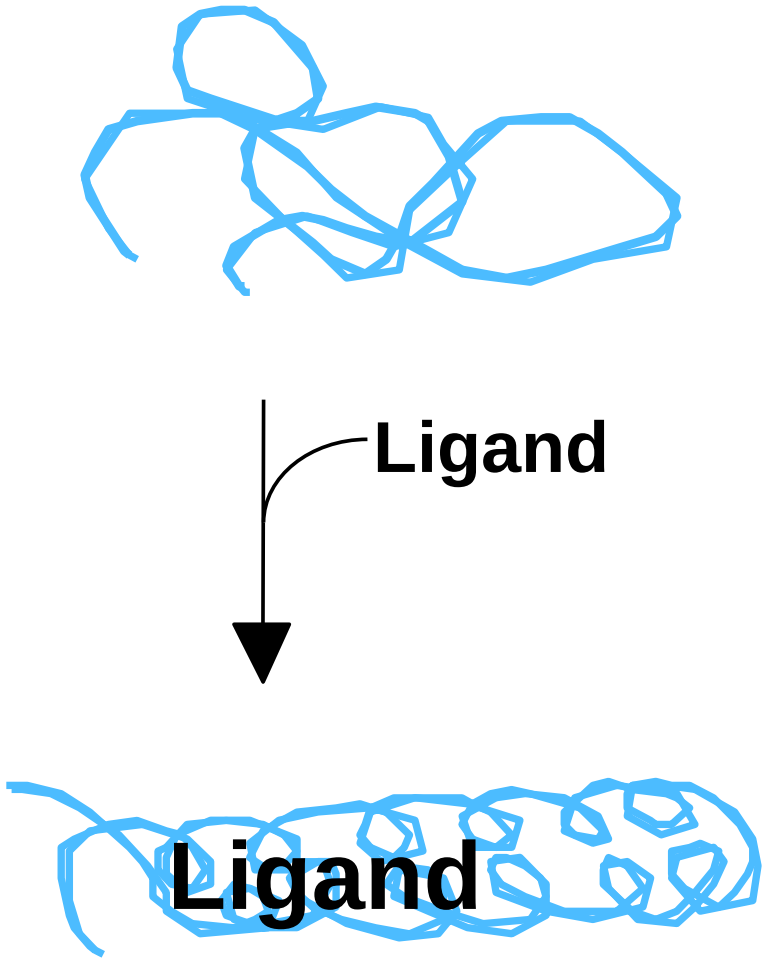


Signaling linked to a binding-specific change in the physical properties of the biopolymer

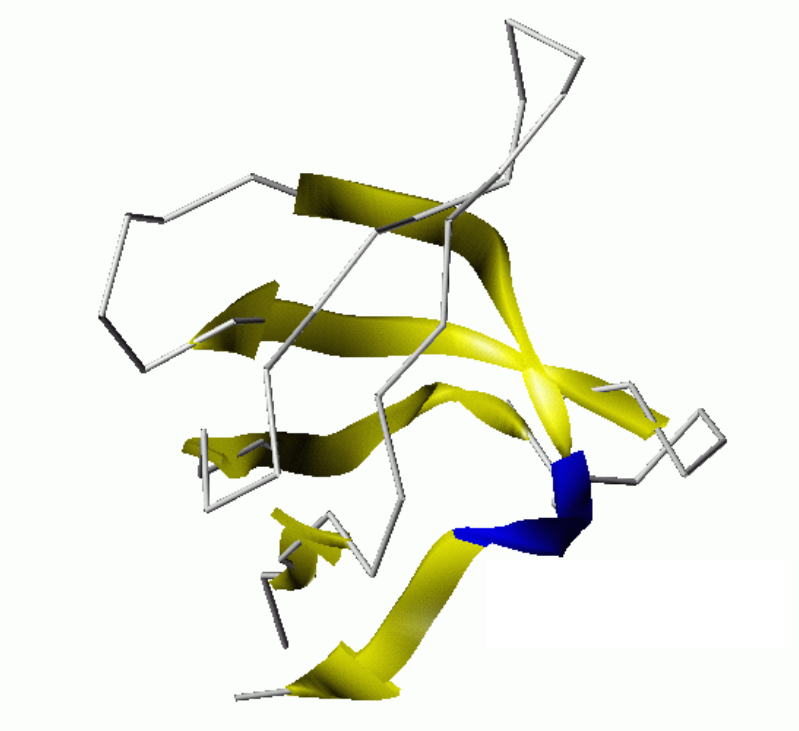
Folding

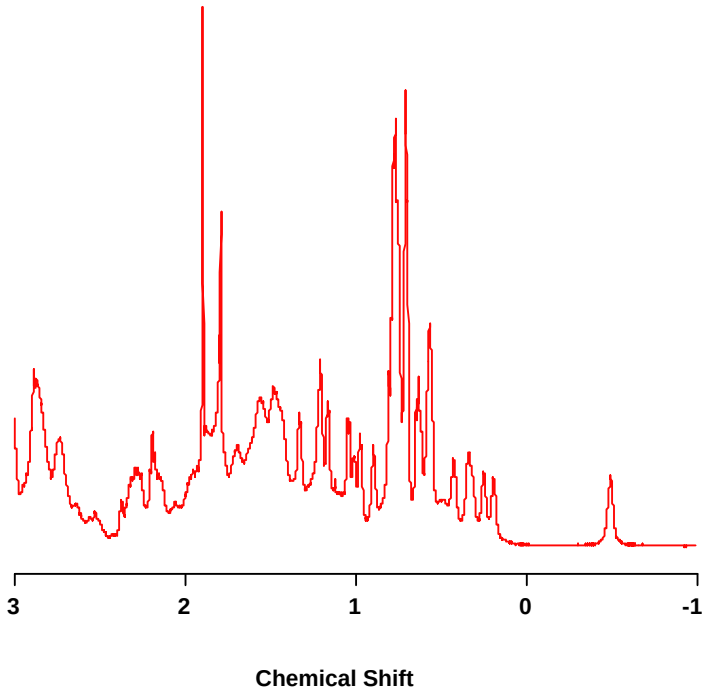


Ligand-induced folding (LIF)

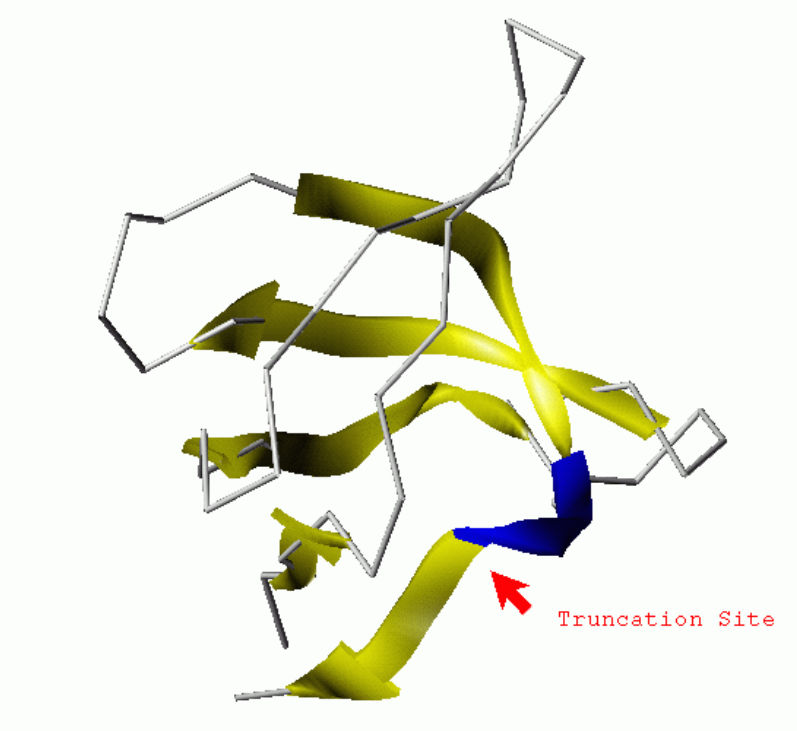


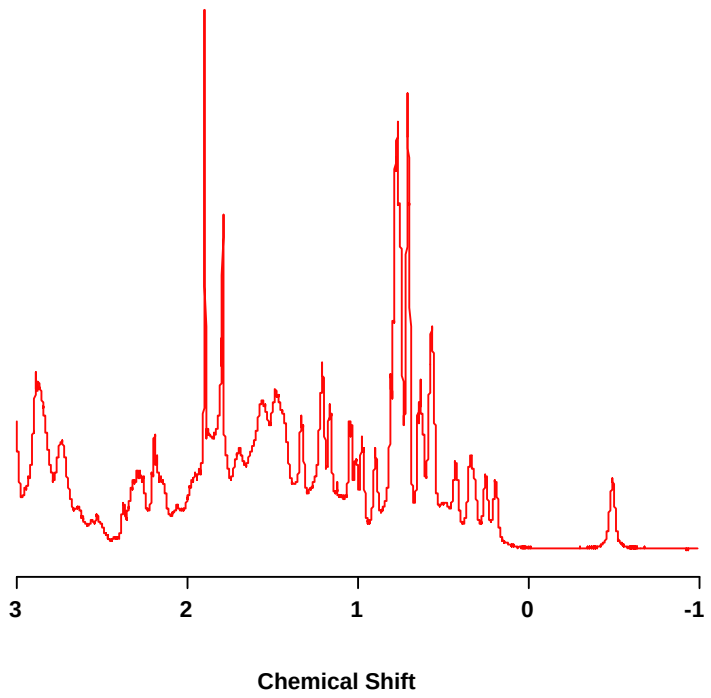
Engineered signal transduction





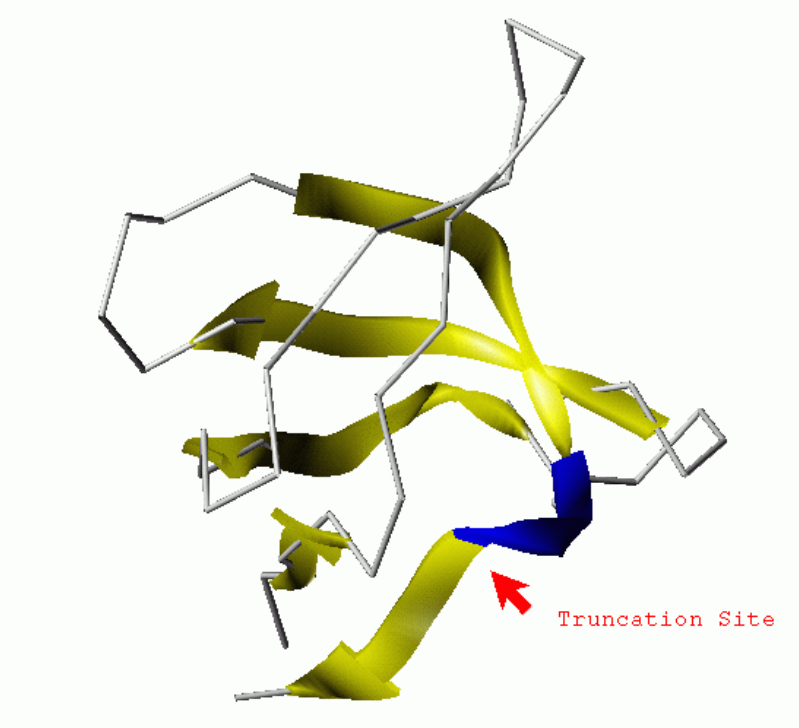
Full-length protein

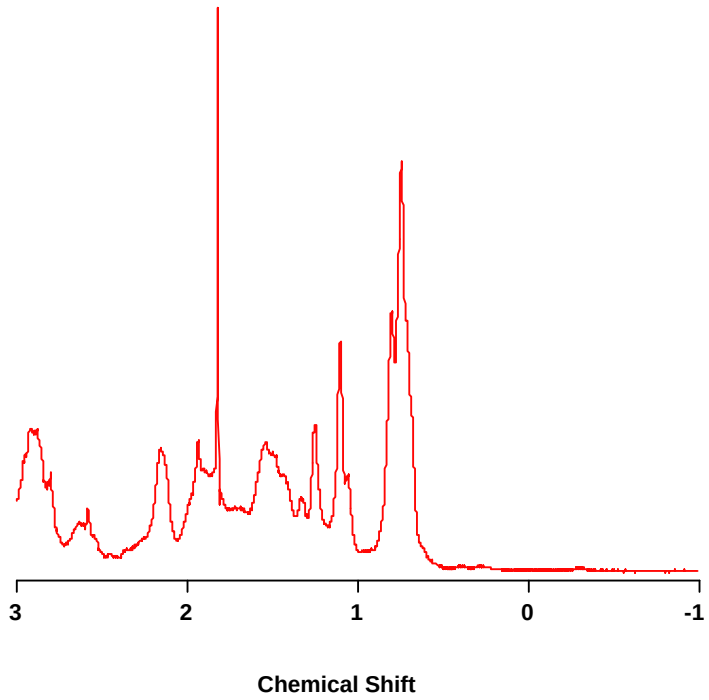




Full-length protein

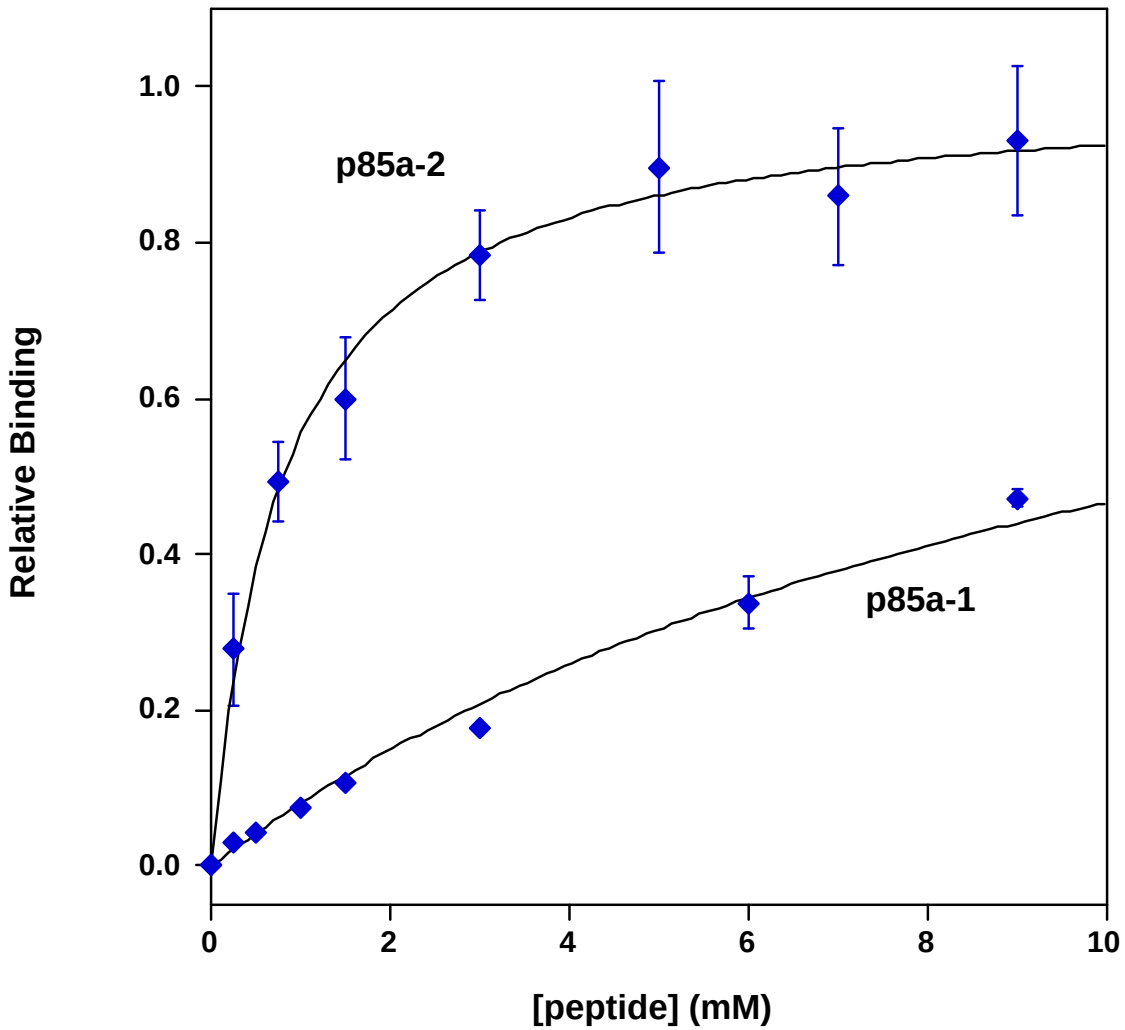
Truncated = Unfolded



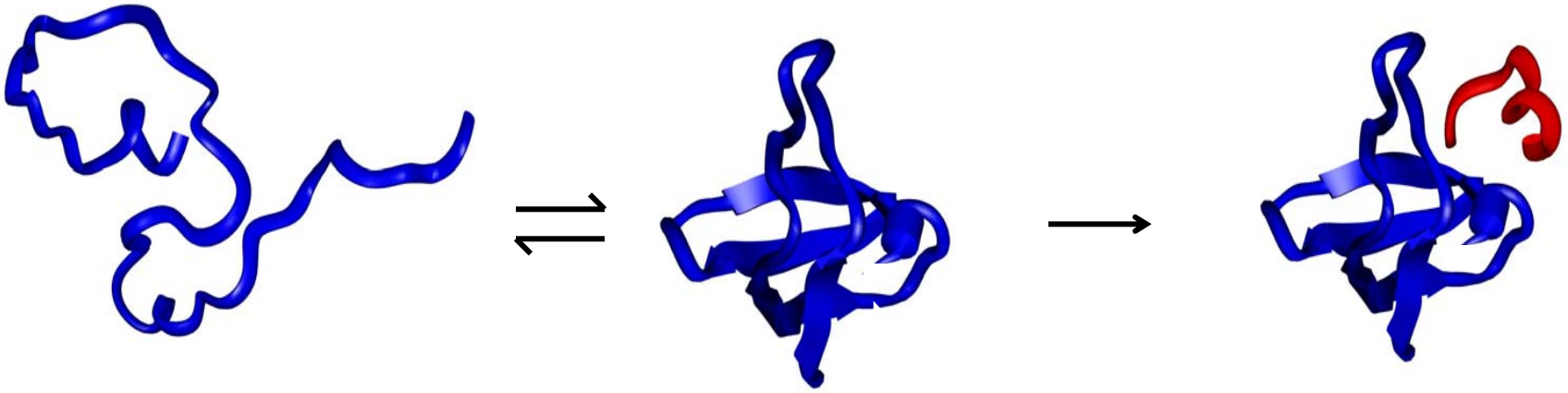


Truncation Mutant

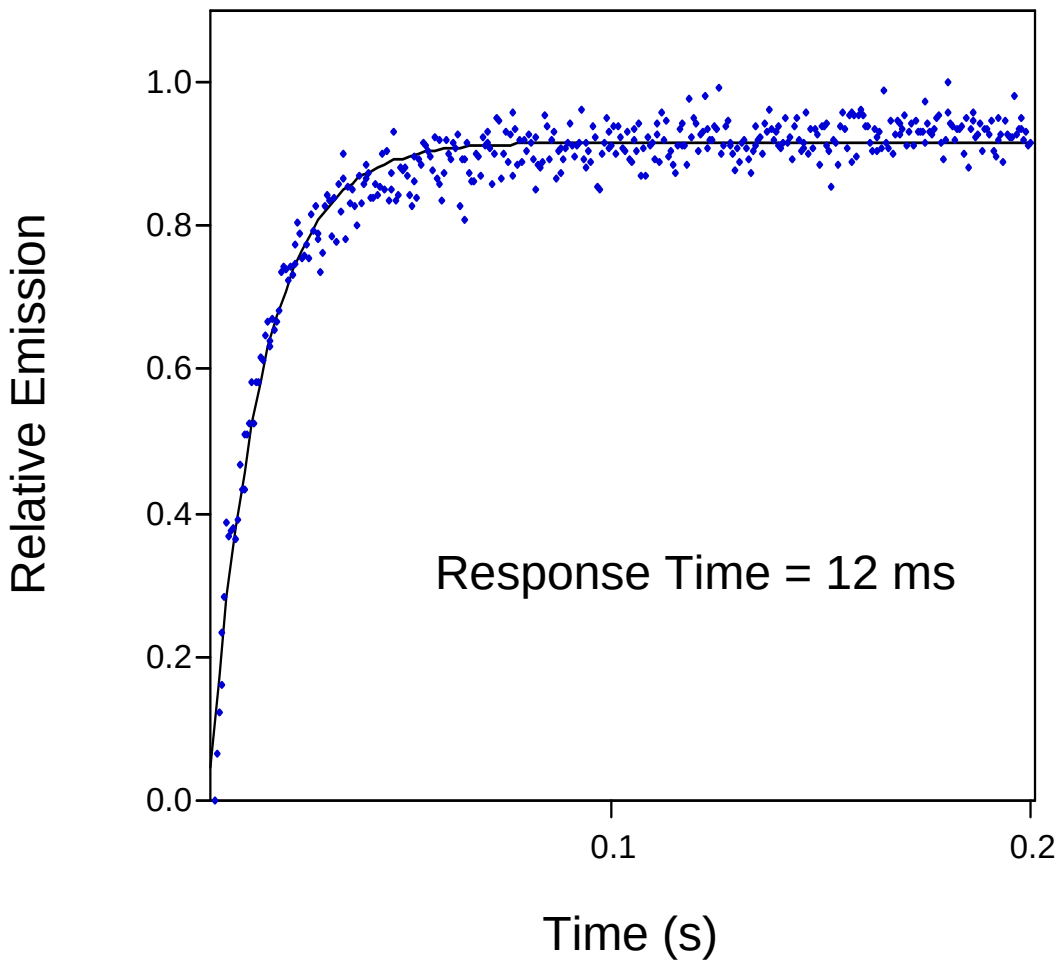
Ligand-specific Folding



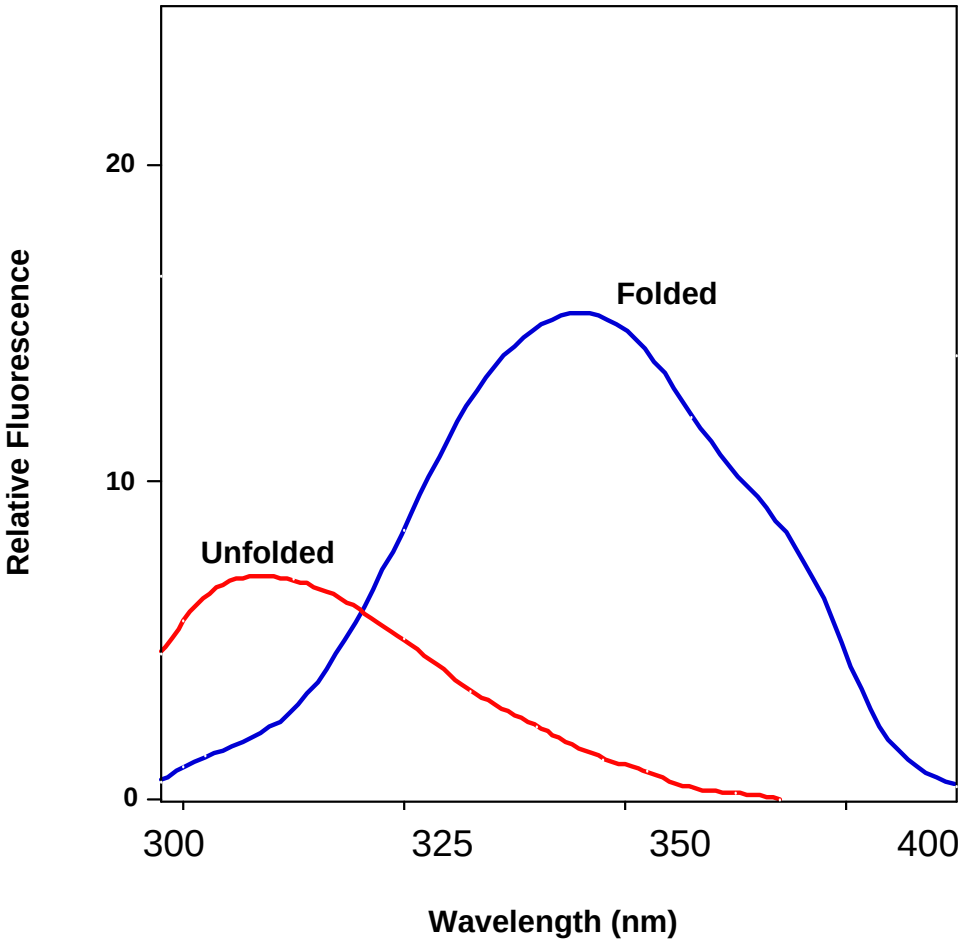
LIF Mechanism



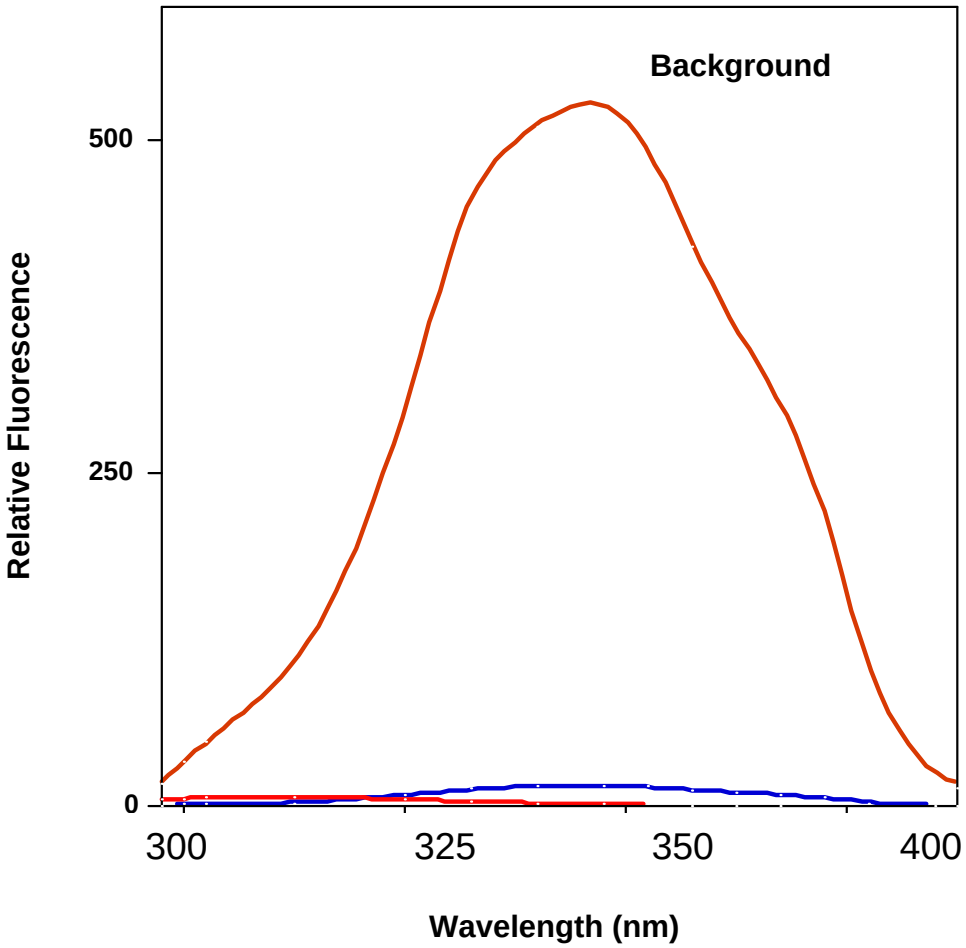
Rapid


Relative Emission
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
Time (s)
Response Time = 12 ms

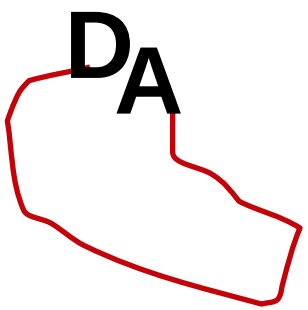
In the Laboratory

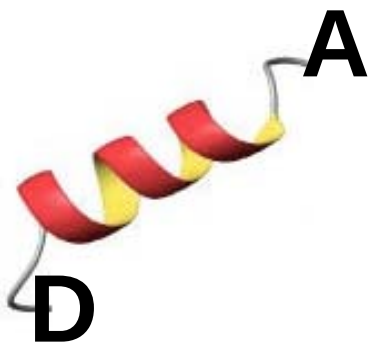


In the Real World



Dynamics-based Signal Transduction

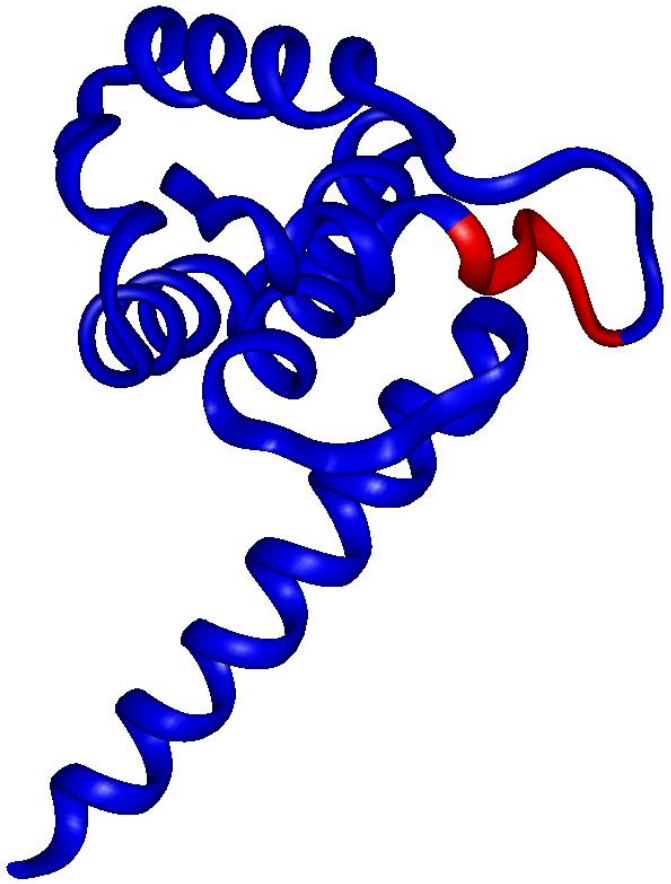




τ_c ~ 10 - 100 ns

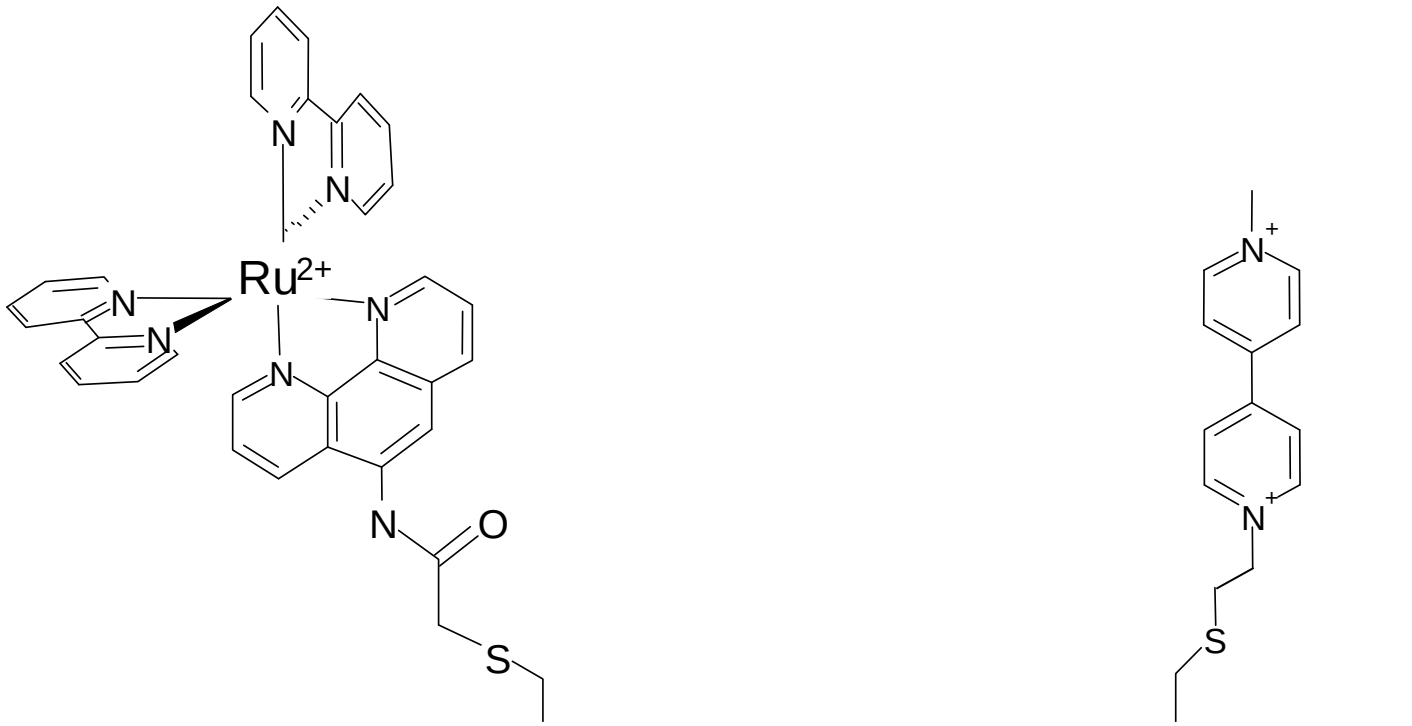
Rigid

p17 Epitope



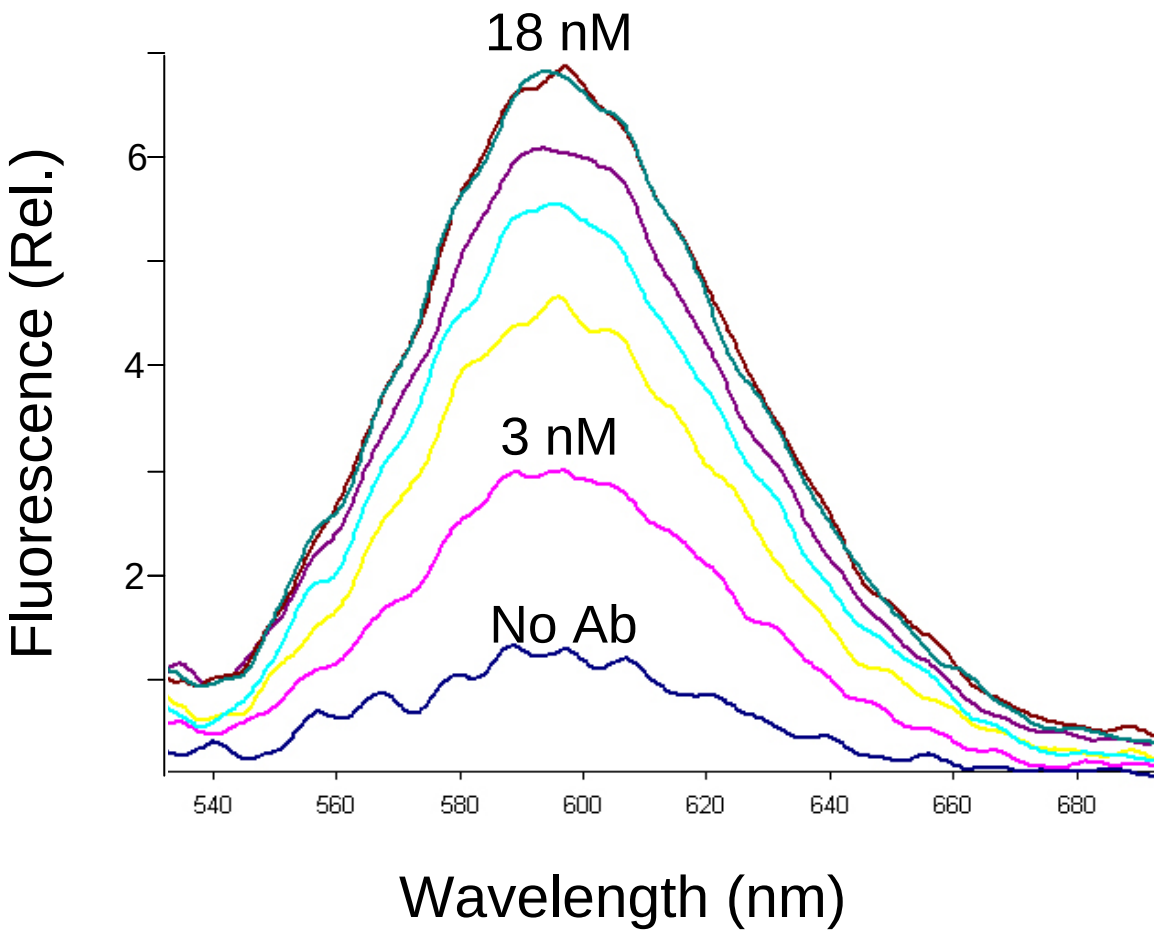
- A six-residue peptide that binds anti-HIV antibodies with nM affinity
- Detection of binding equates to detection of HIV infection

Peptide Beacon

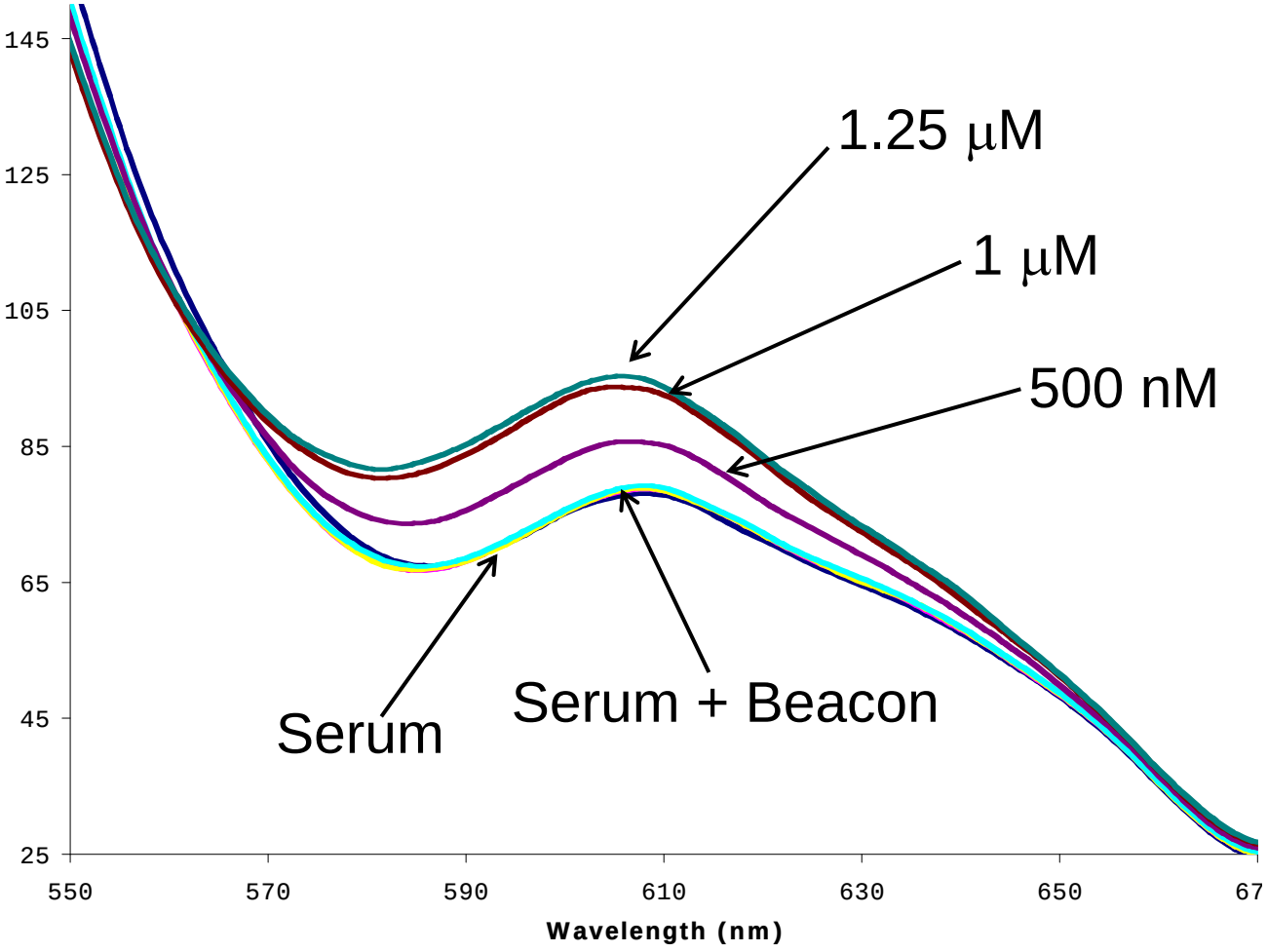


Ac-CGKIRLRPGC-OH

Anti-HIV Antibody Detection

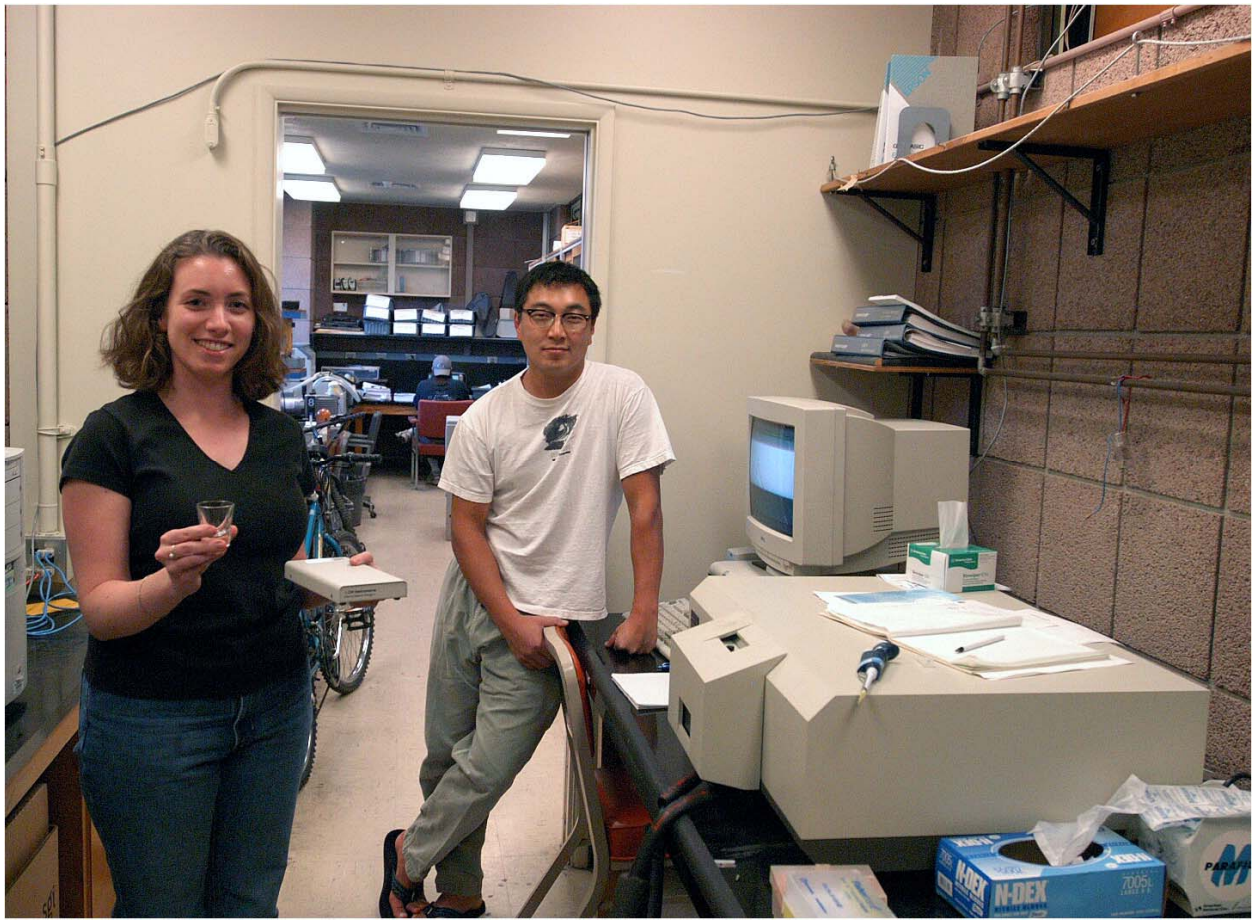


Antibody Detection in Serum

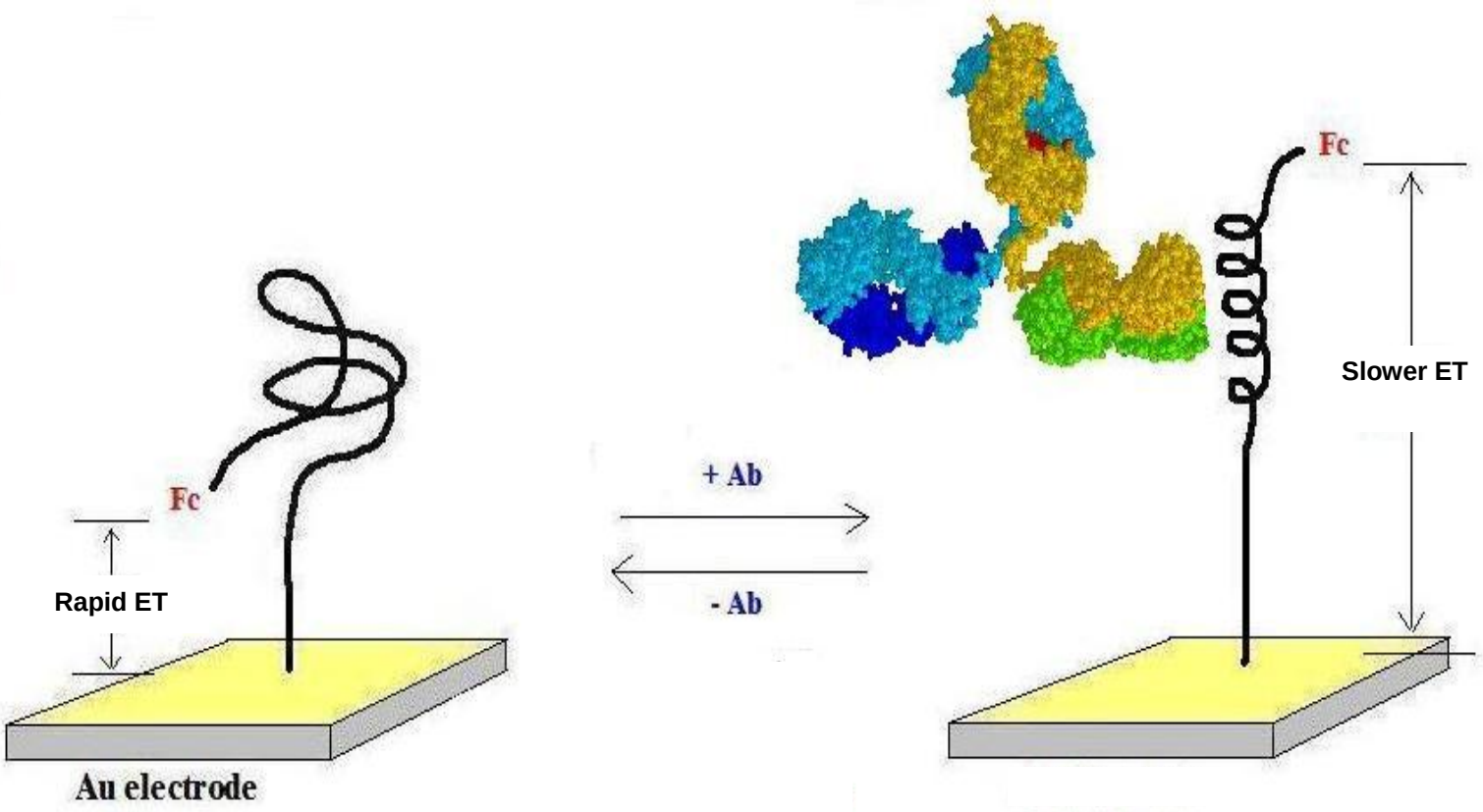


Electrochemical Biosensors

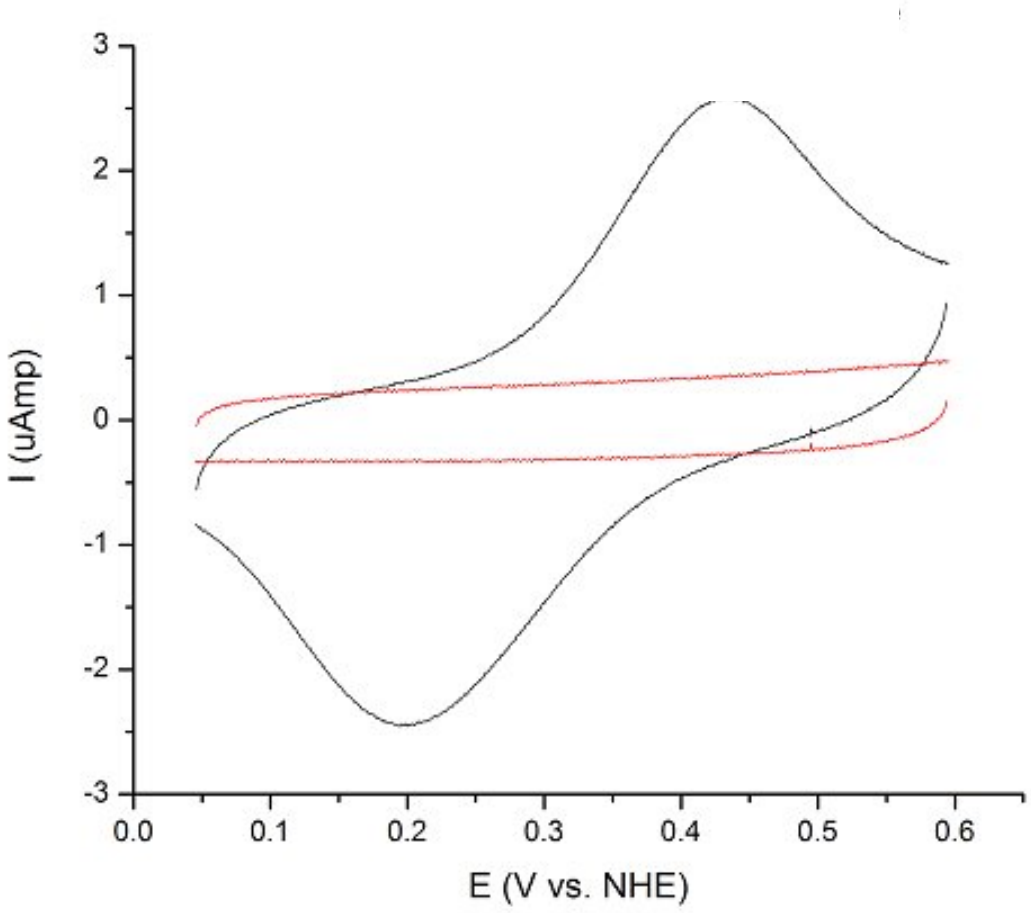
- **Extremely low background**
- **Low mass, power, volume**
- **Low cost, mass production**
- **Parallelization**



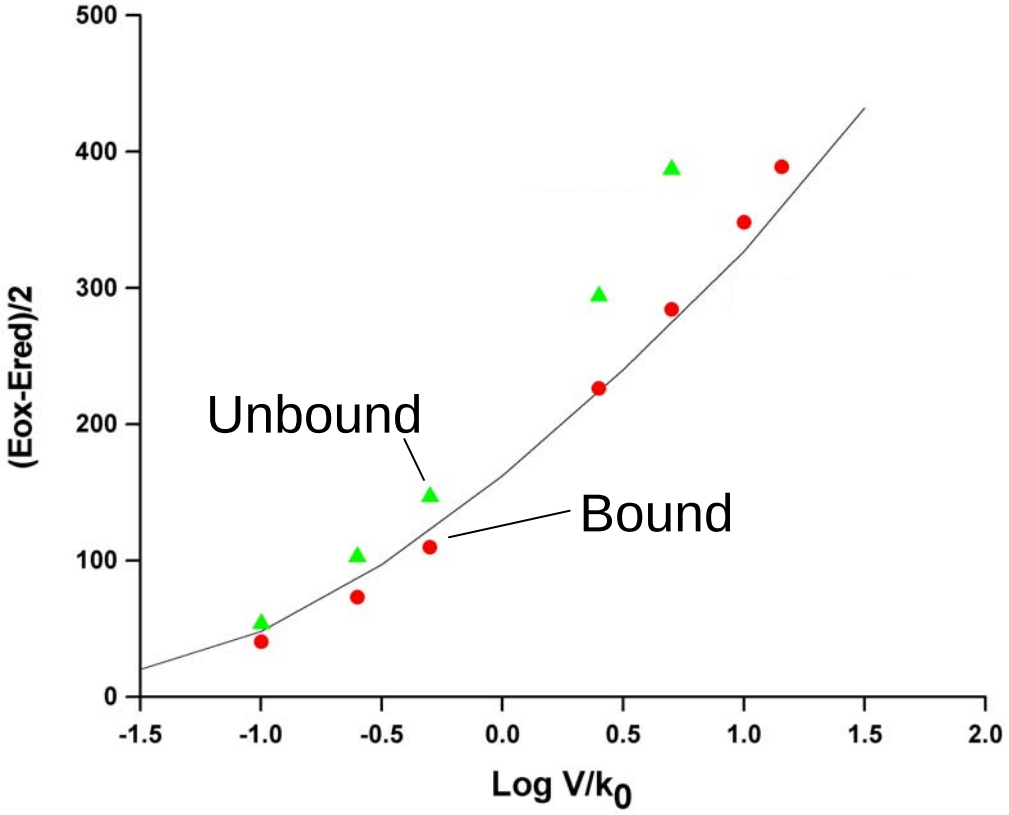
Electronic Peptide Beacons



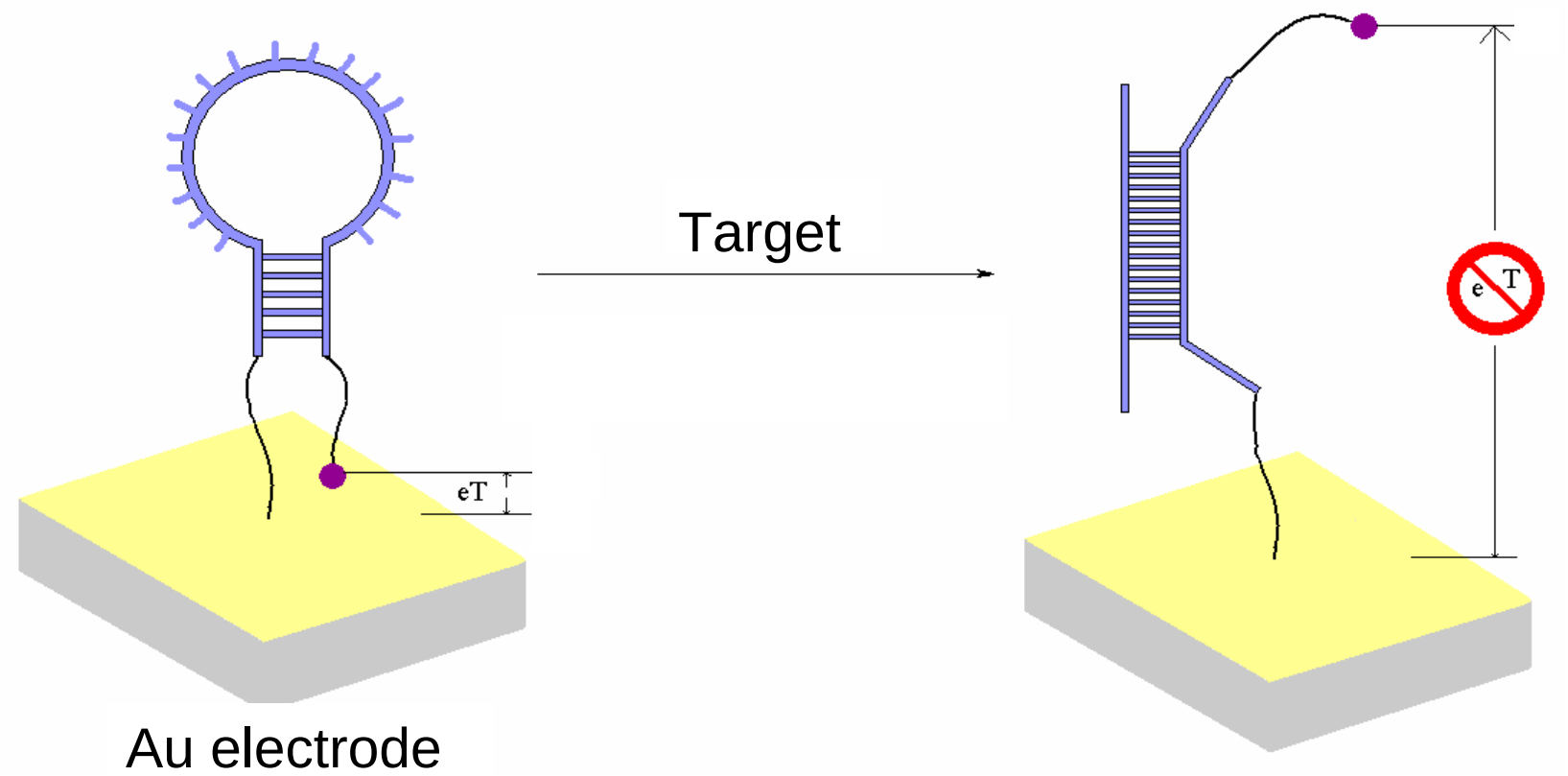
Cyclic Voltammetry



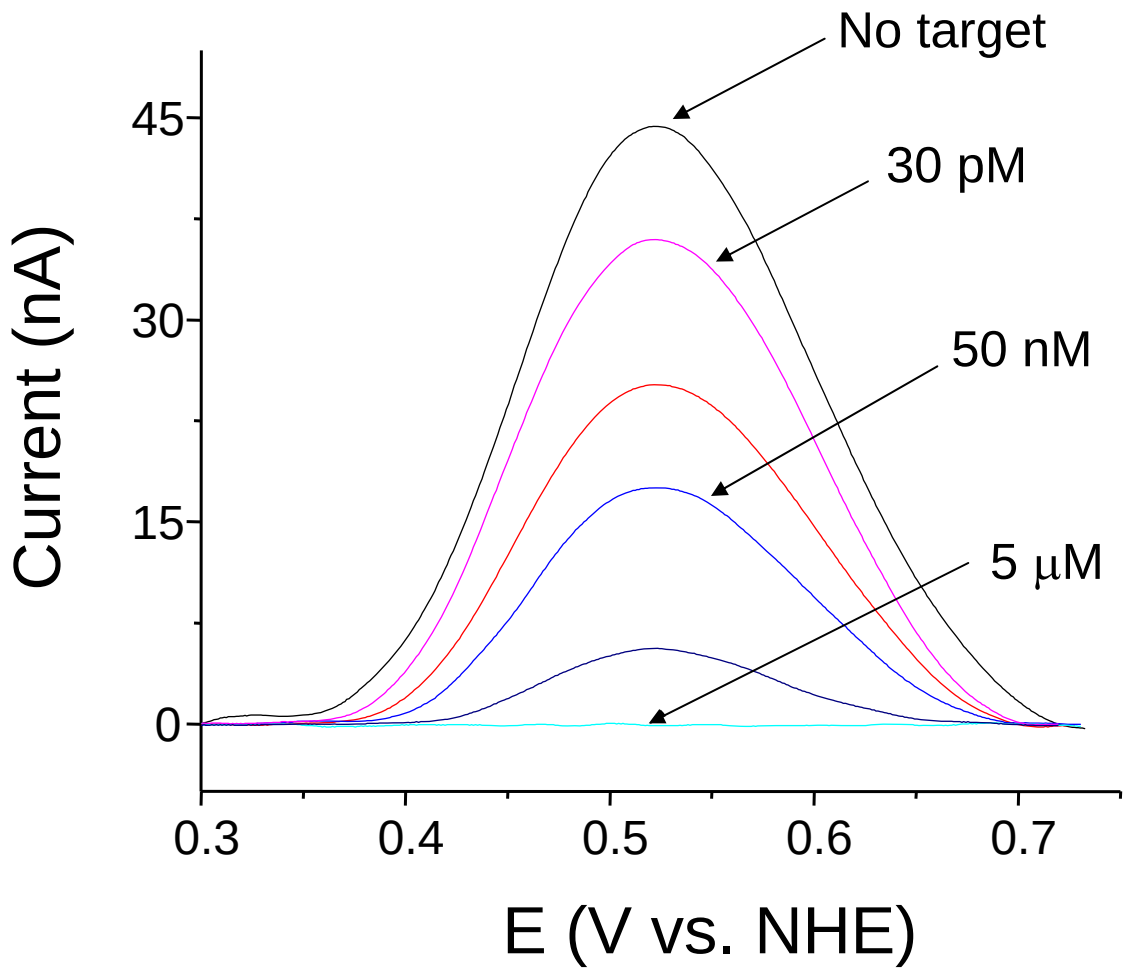
Electronic Peptide Beacons



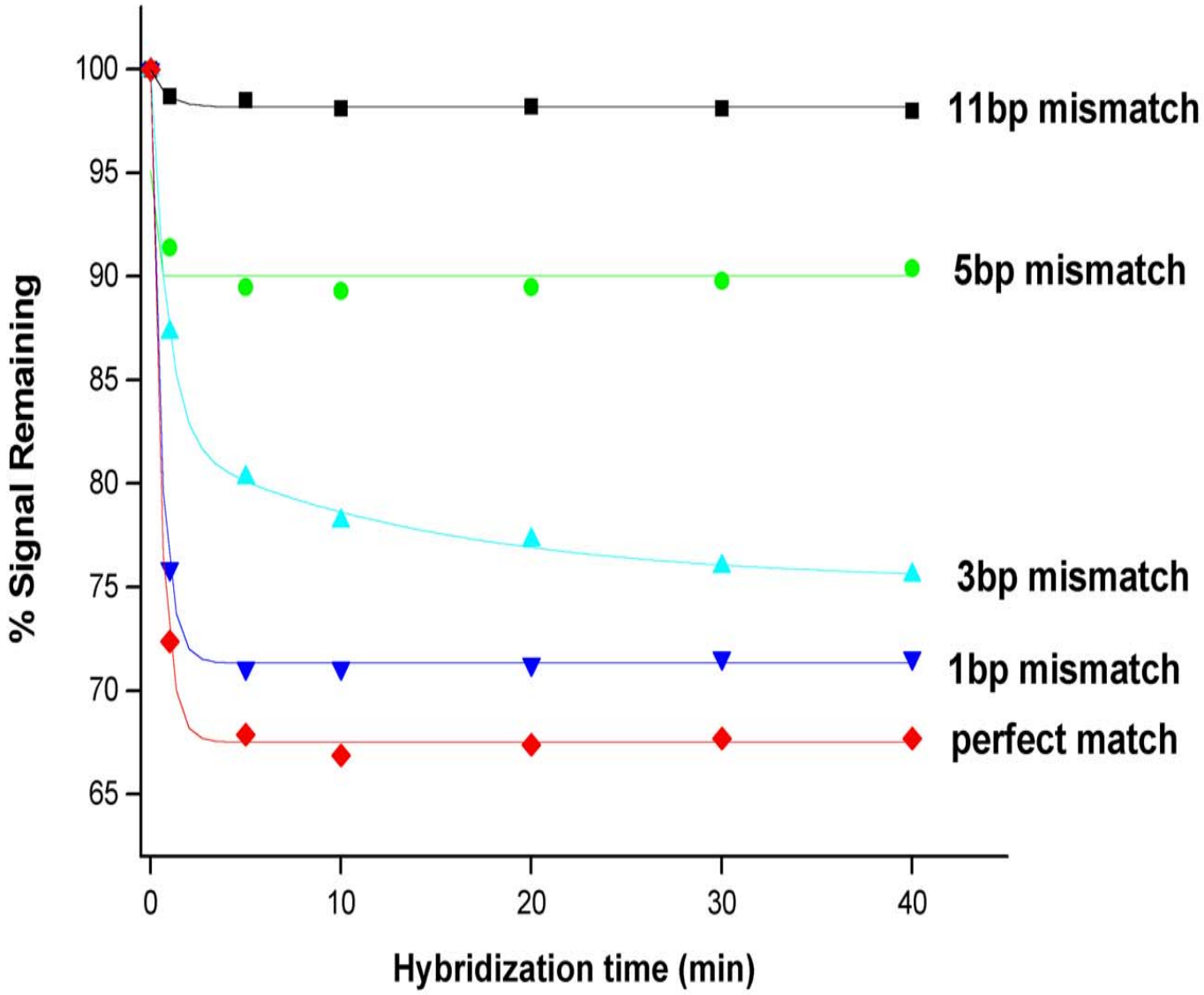
E-DNA



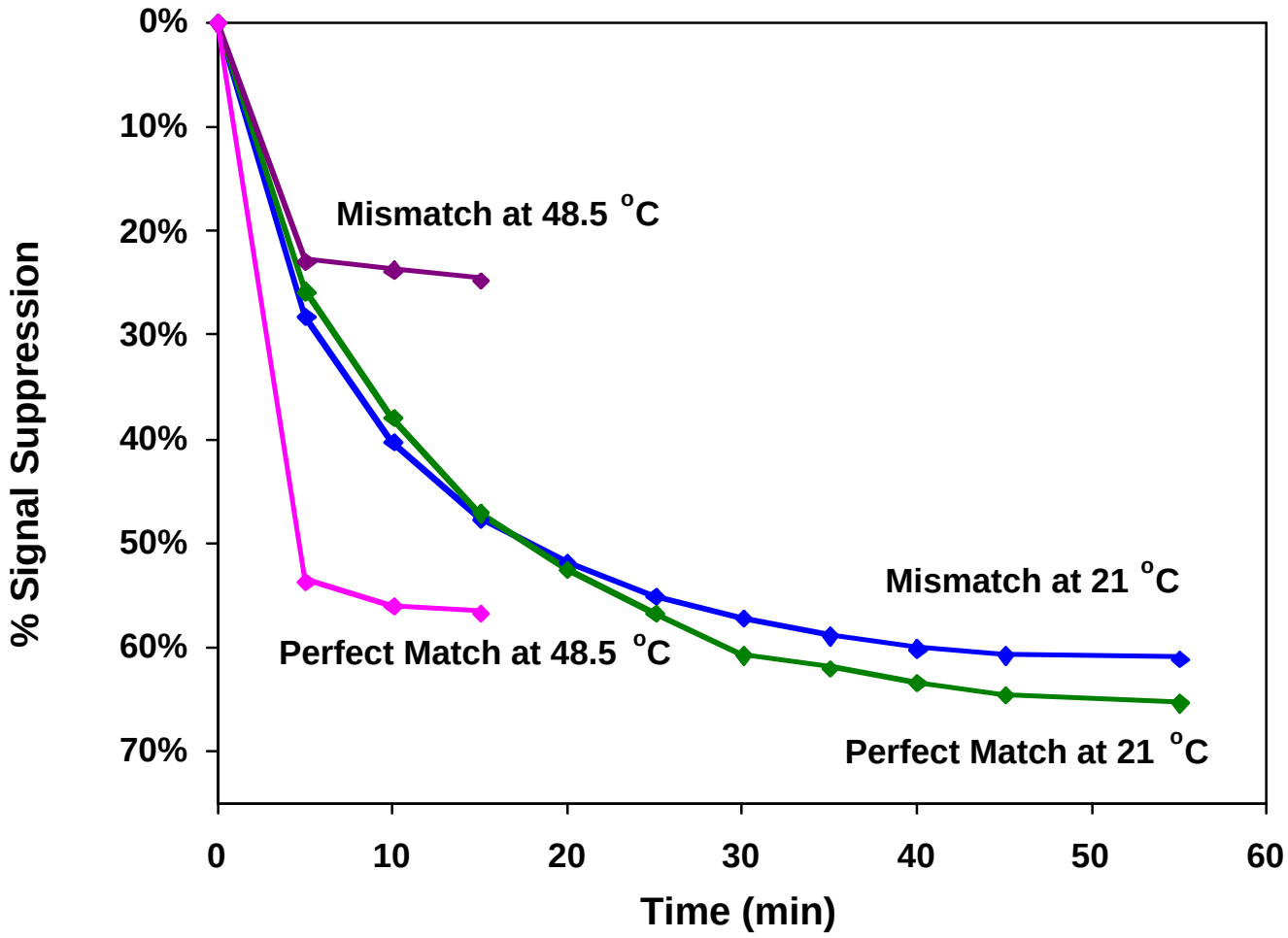
Sensitive


No target
30 pM
50 nM
5 μM
45
30
15
0
Current (nA)
0.3
0.4
0.5
0.6
0.7
E (V vs. NHE)

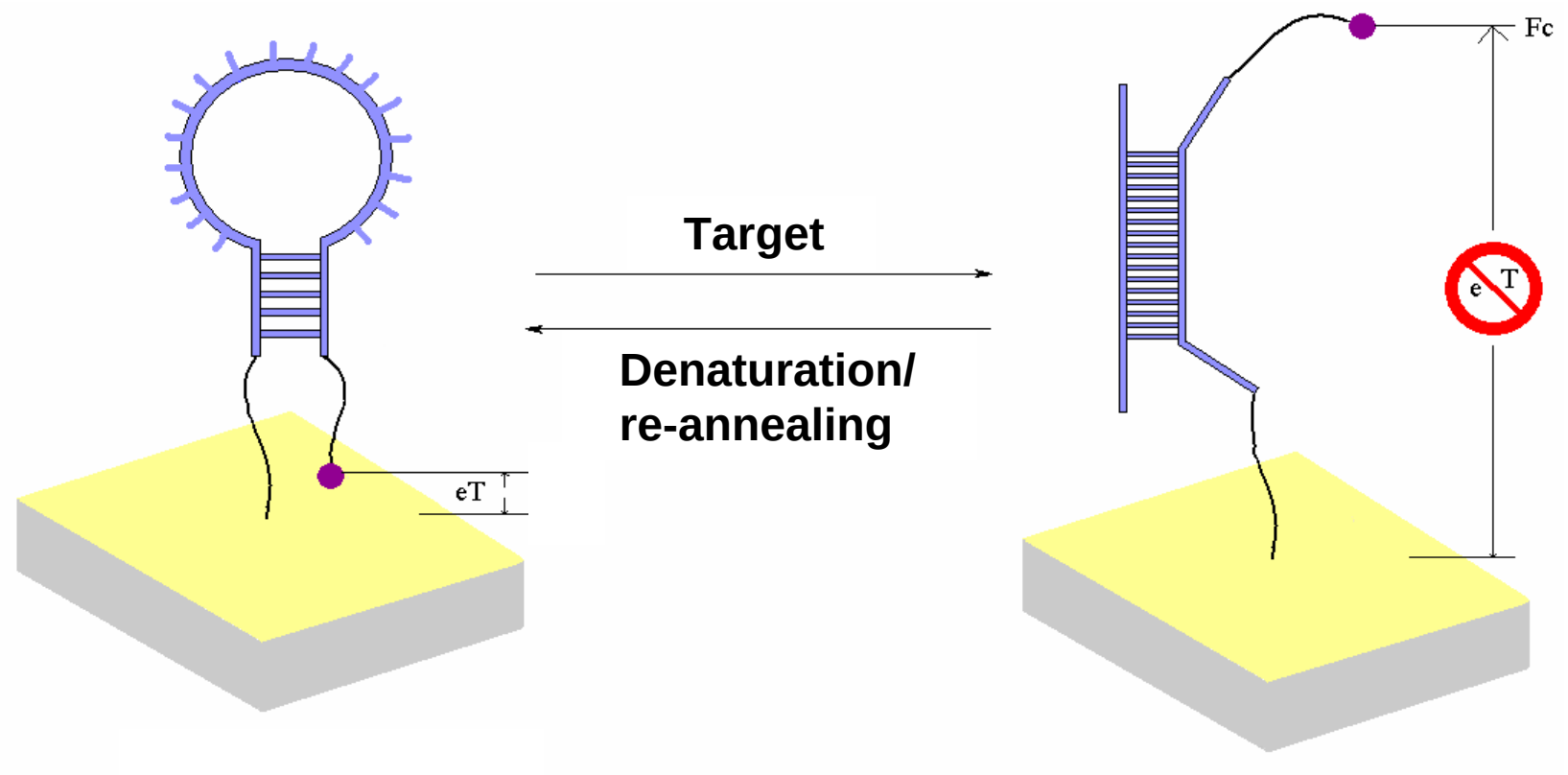
Rapid and Specific



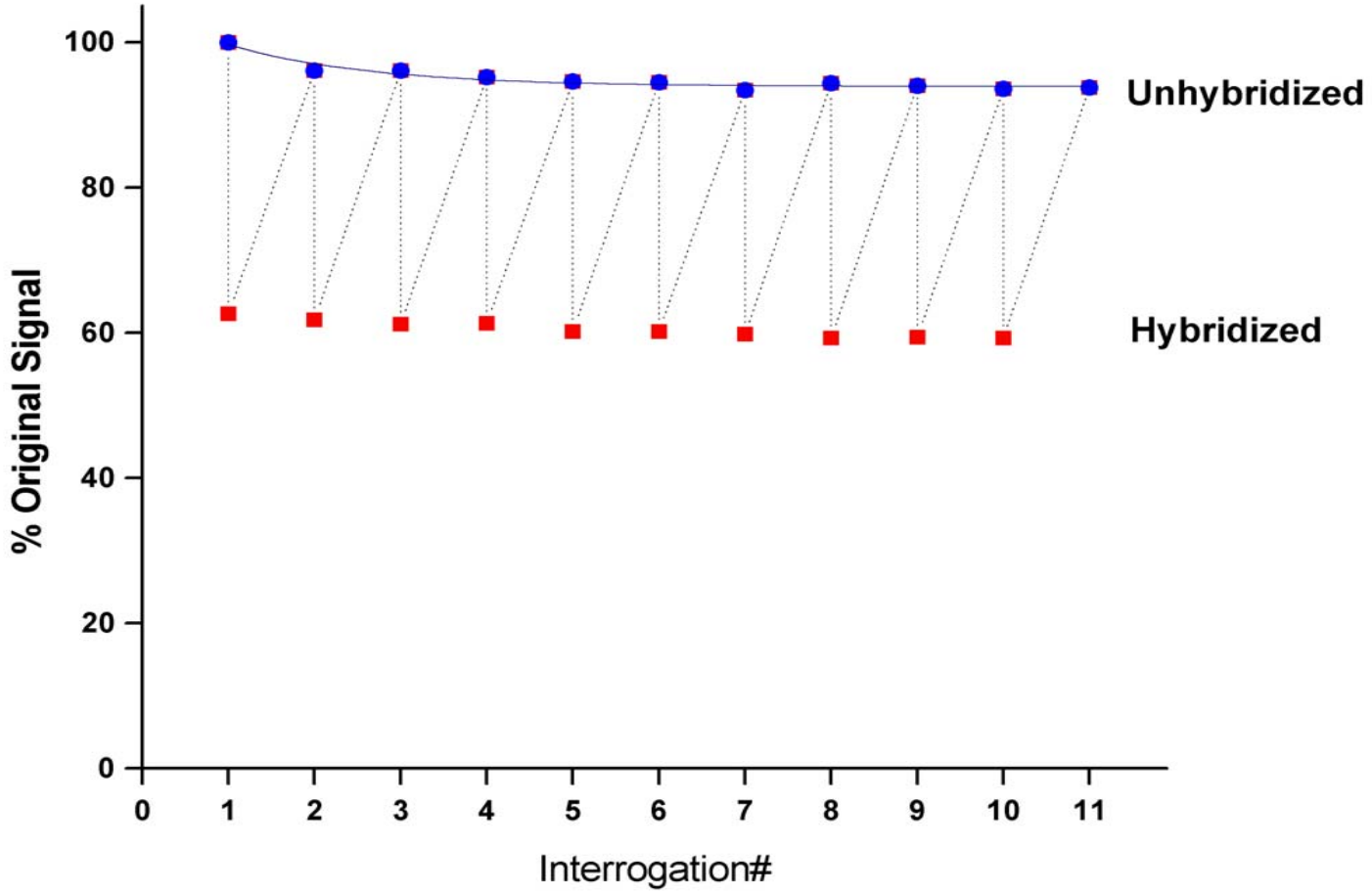
Rapid and Specific



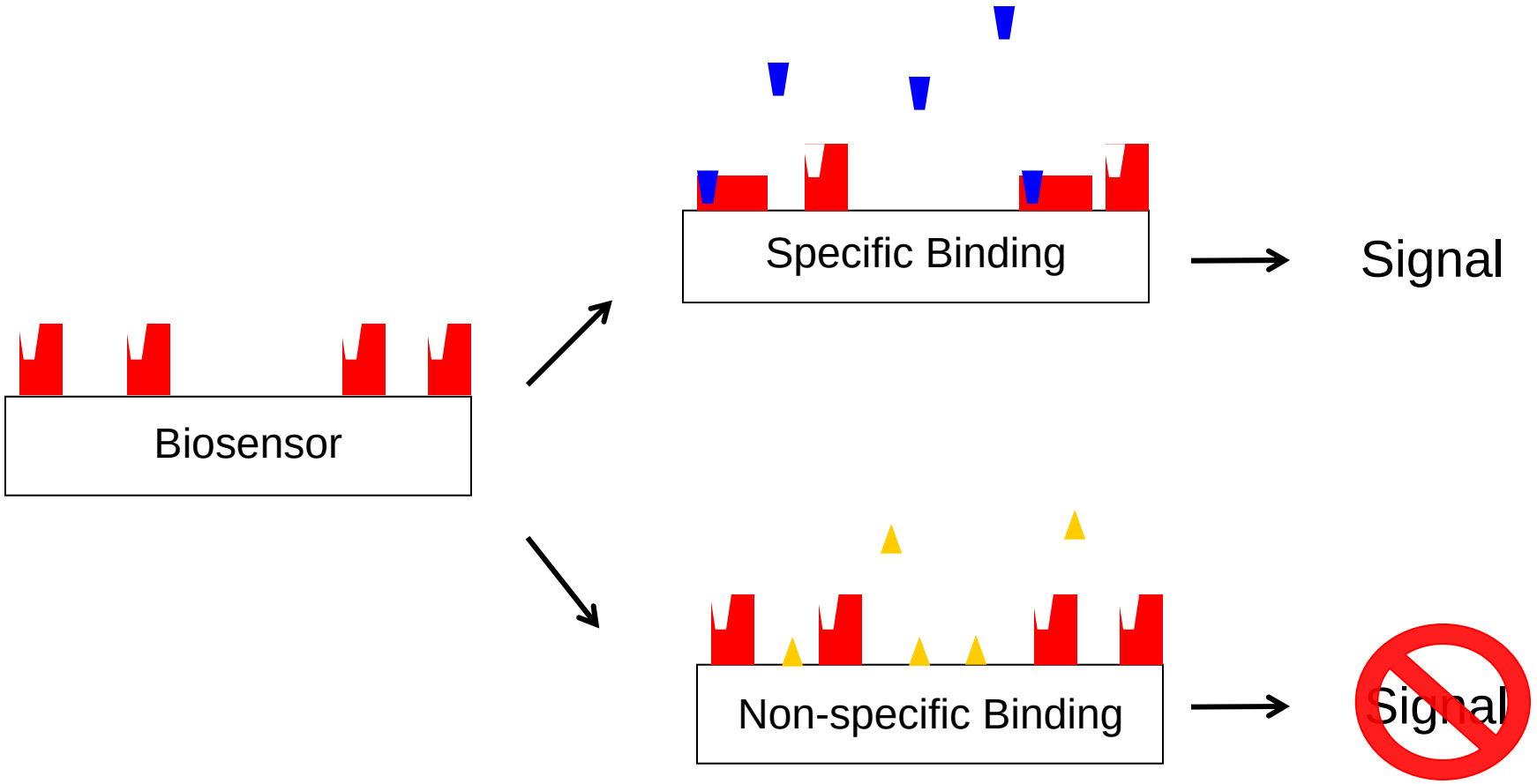
Reagentless, …

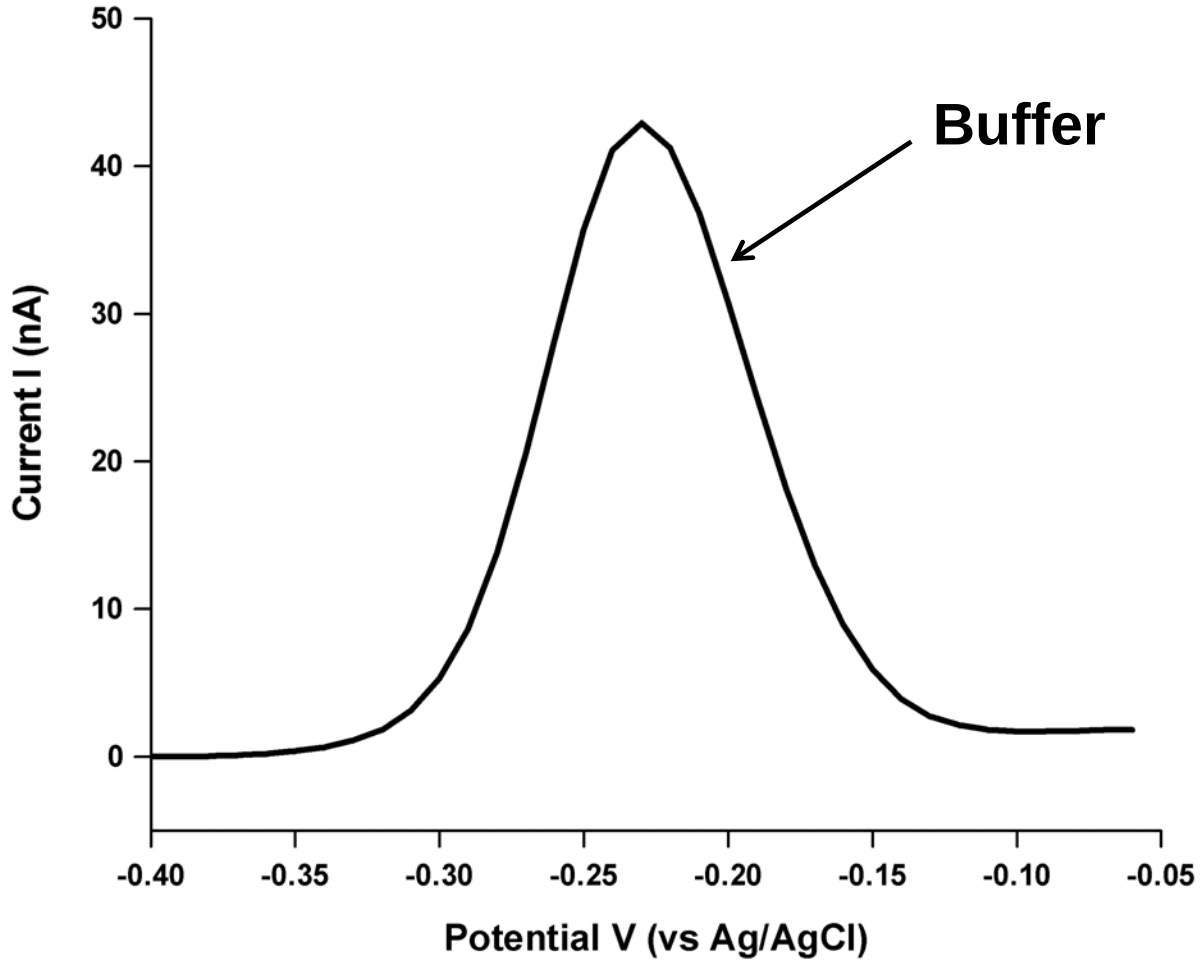


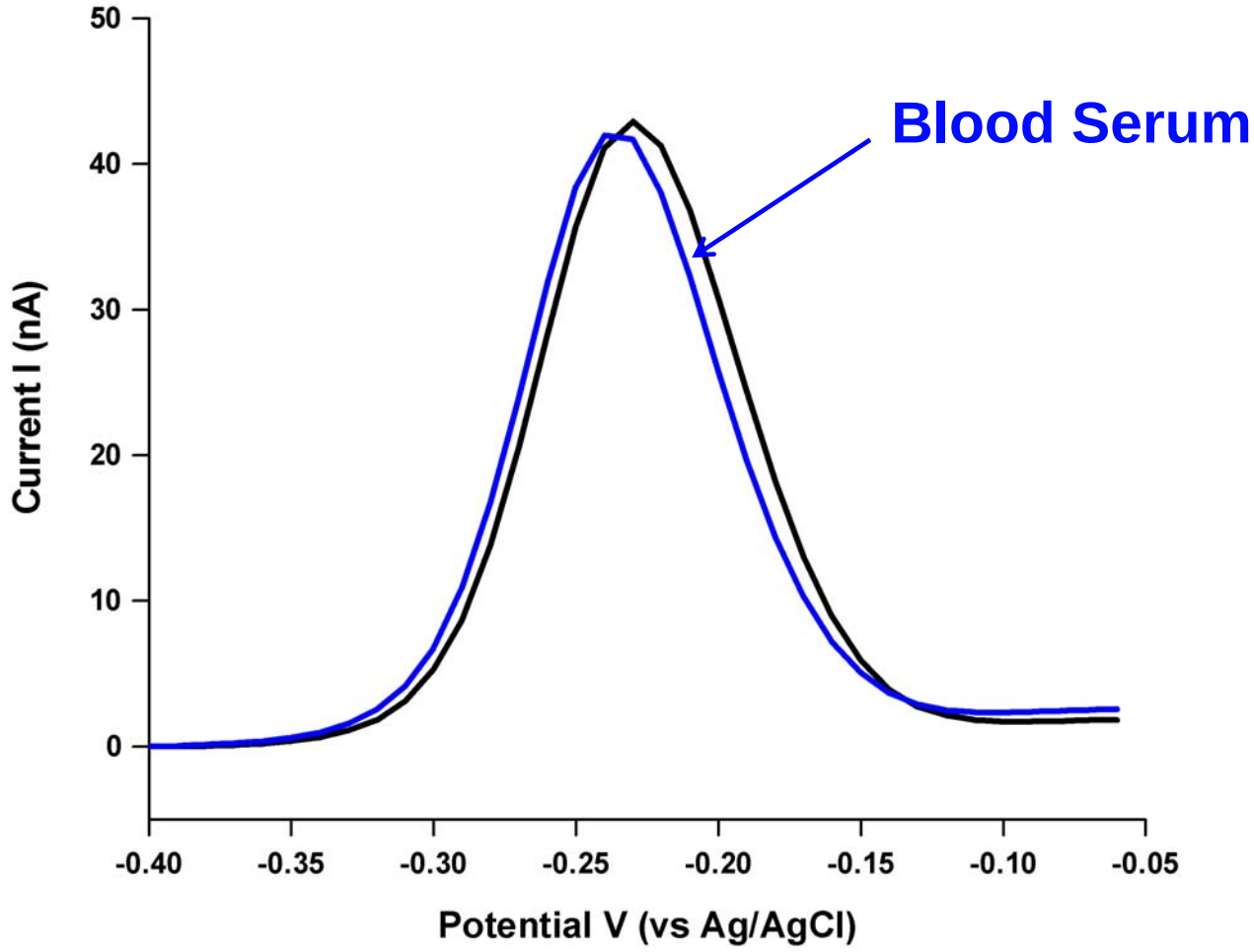
…Reusable, Reproducible

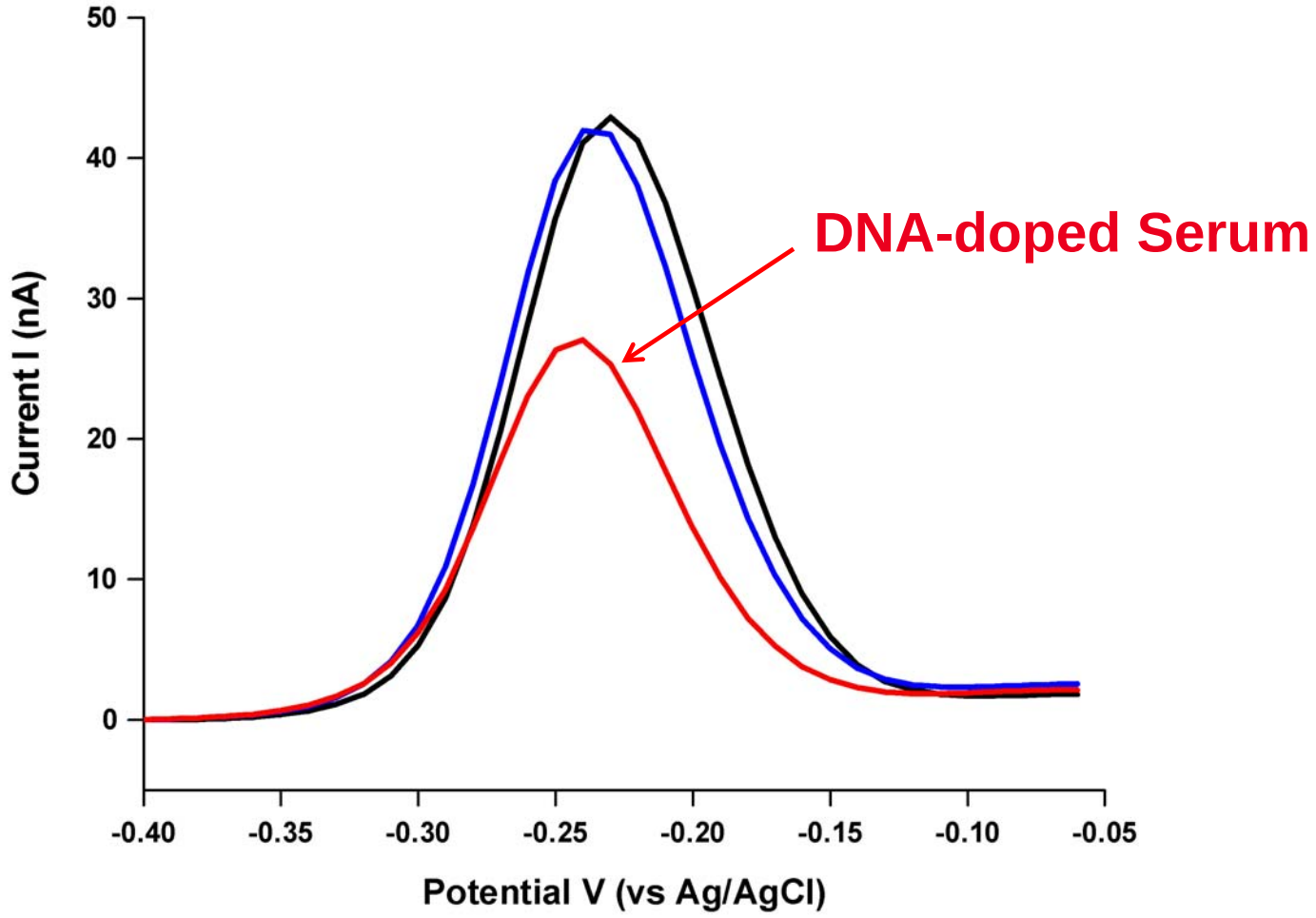


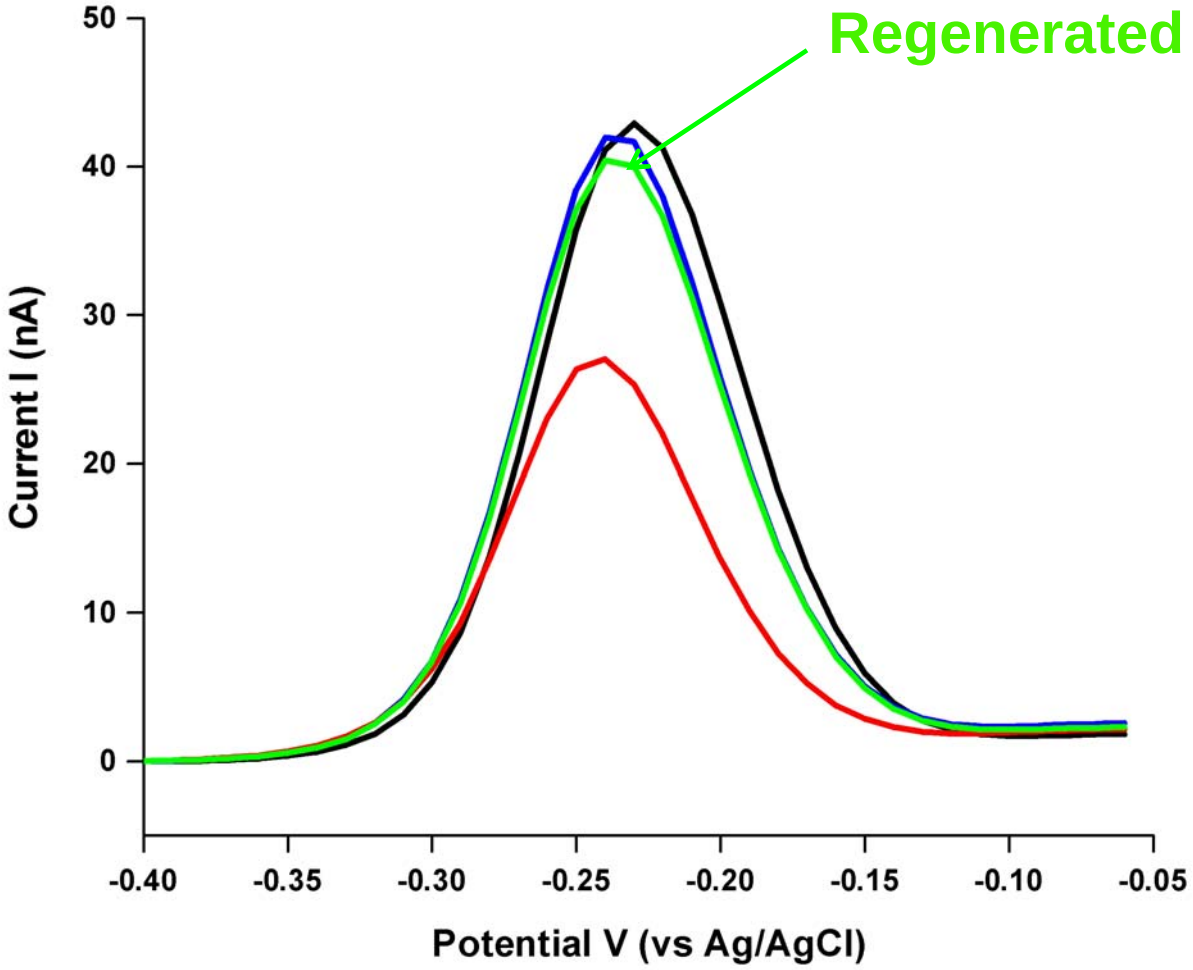
Selective?

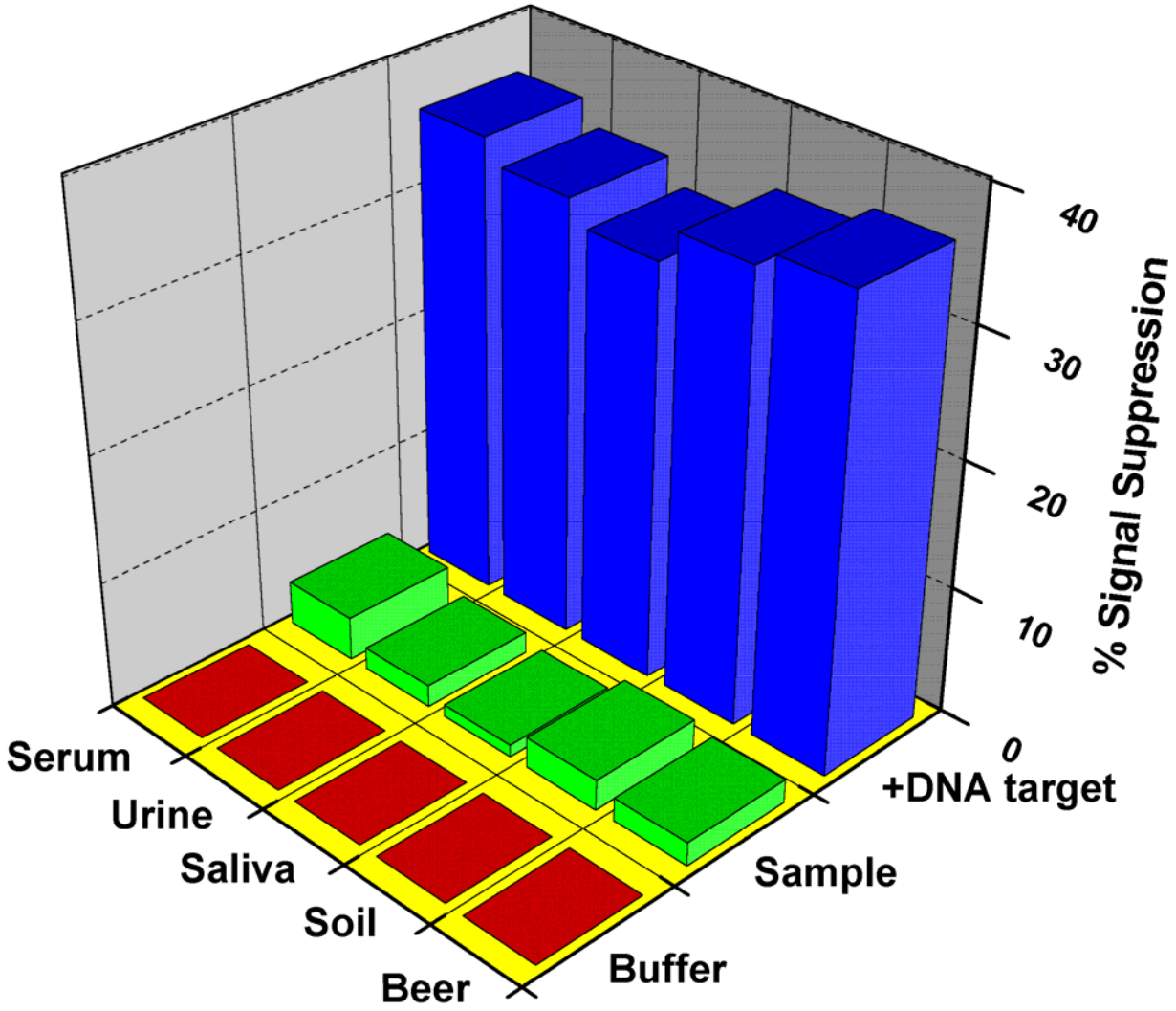

Specific Binding
Signal
Biosensor
Non-specific Binding
Signal

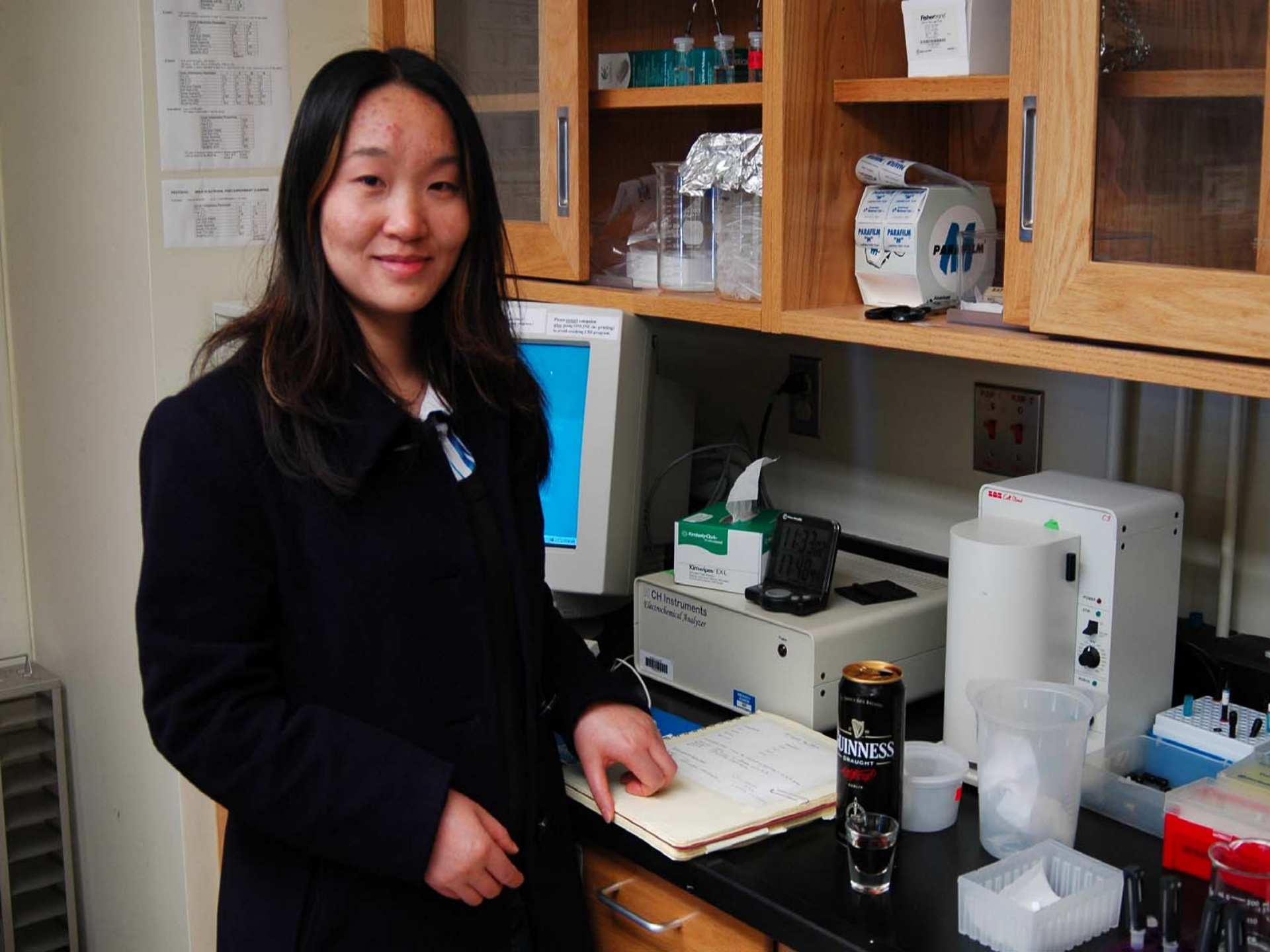

Buffer
Current I (nA)
50
40
30
20
10
0
-0.40
-0.35
-0.30
-0.25
-0.20
-0.15
-0.10
-0.05
Potential V (vs Ag/AgCl)


Blood Serum
Current I (nA)
50
40
30
20
10
0
-0.40
-0.35
-0.30
-0.25
-0.20
-0.15
-0.10
-0.05
Potential V (vs Ag/AgCl)

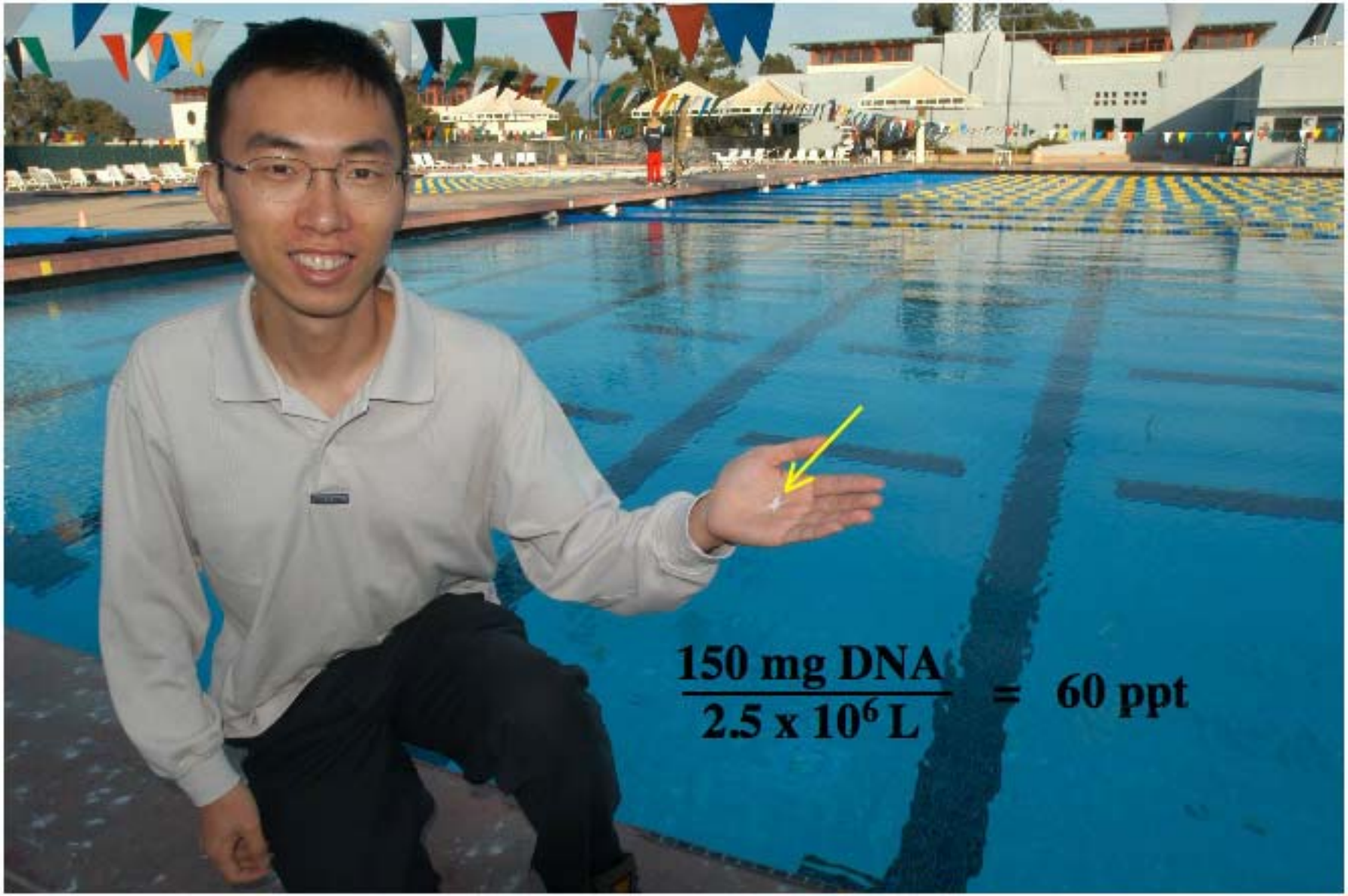

DNA-doped Serum
Current I (nA)
50
40
30
20
10
0
-0.40
-0.35
-0.30
-0.25
-0.20
-0.15
-0.10
-0.05
Potential V (vs Ag/AgCl)


Regenerated
Current I (nA)
50
40
30
20
10
0
-0.40
-0.35
-0.30
-0.25
-0.20
-0.15
-0.10
-0.05
Potential V (vs Ag/AgCl)

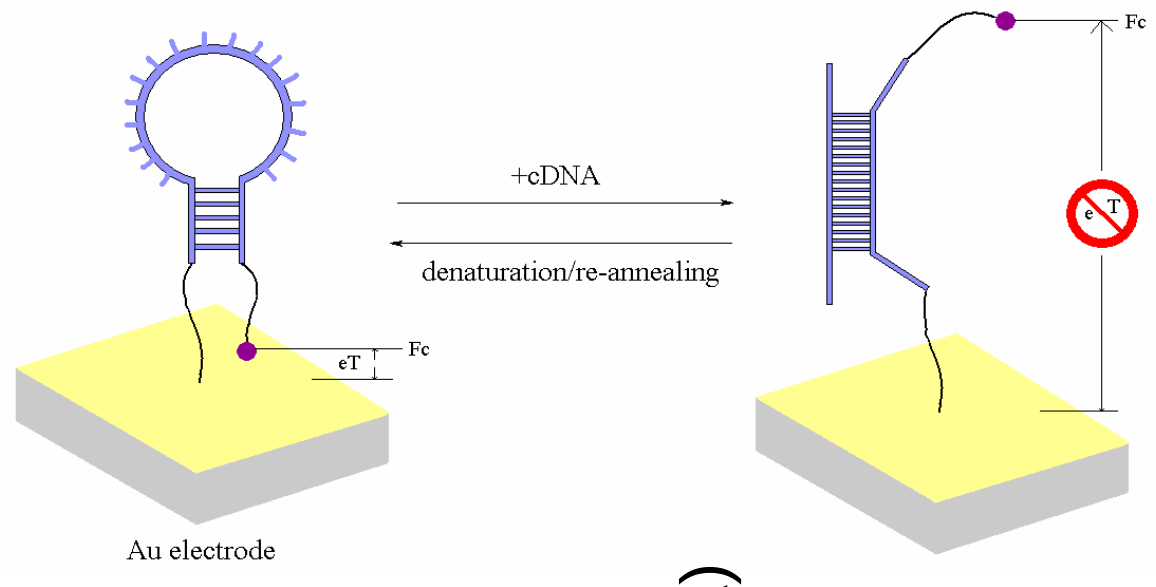

40
30
20
10
0
% Signal Suppression
Serum
Urine
Saliva
Soil
Beer
Buffer
Sample
+DNA target

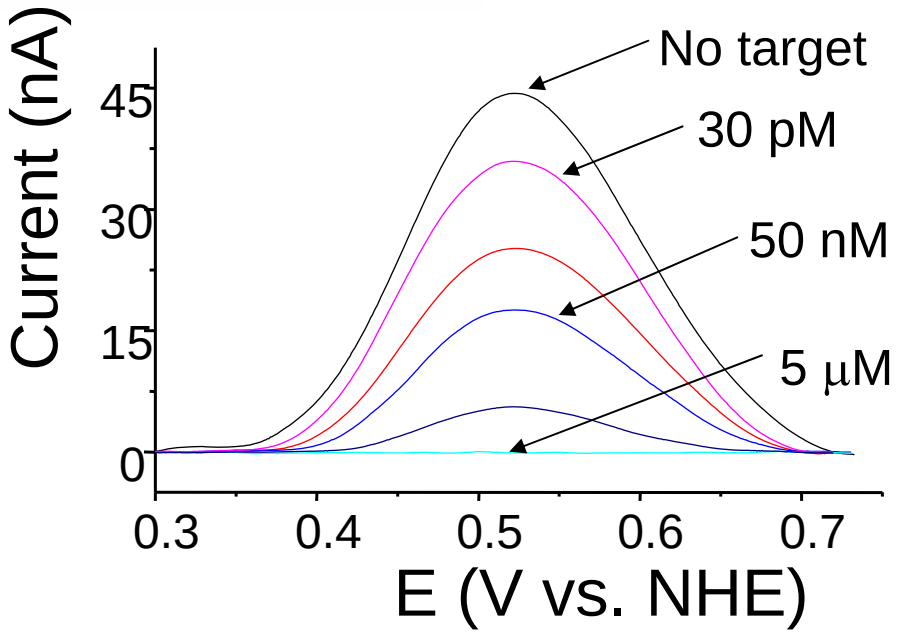

PARAFILM "M"
CH Instruments
Electrochemical Analyzer
Kimwipes EXL
GUINNESS
DRAUGHT

10 pM

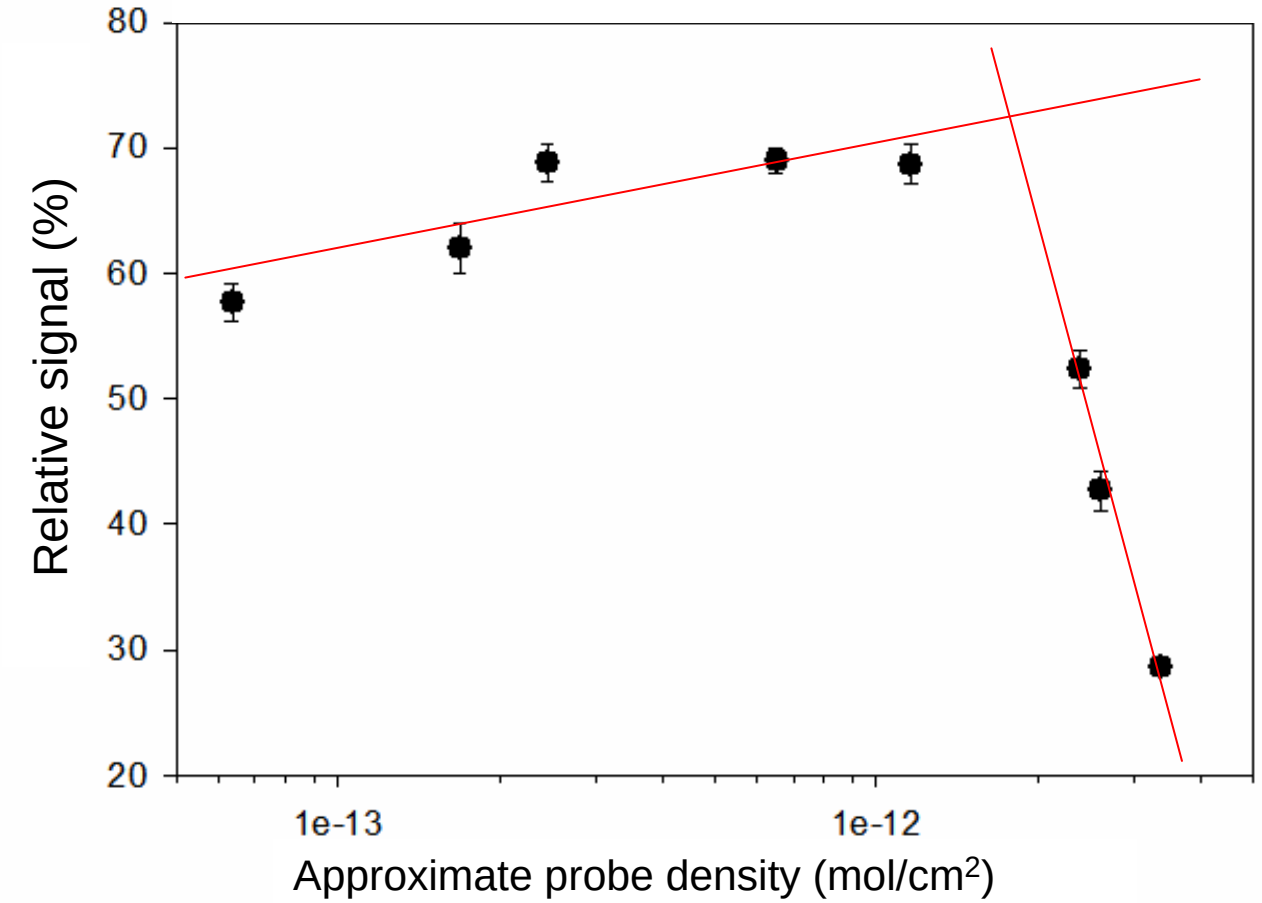


Signal-Off

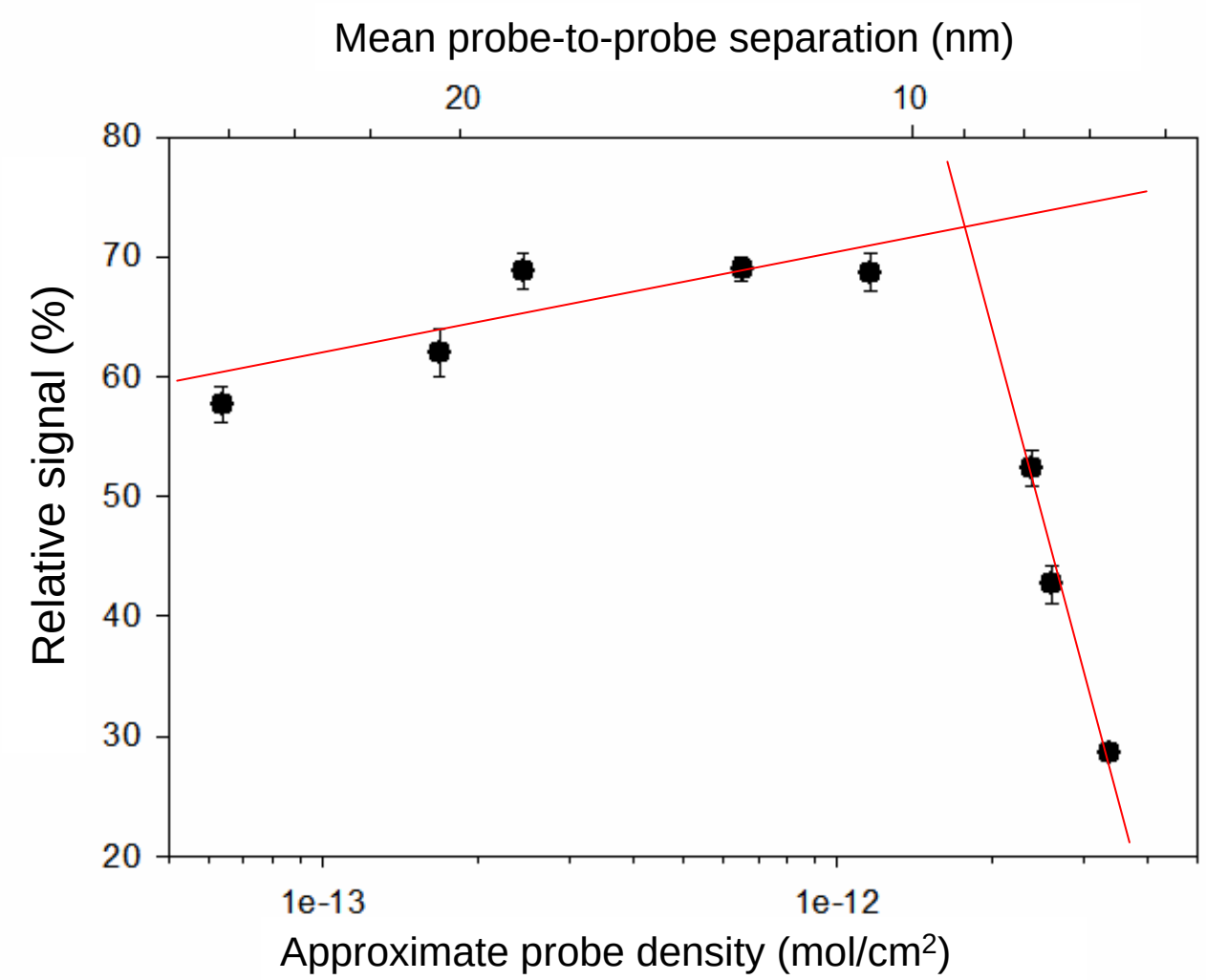

+cDNA
denaturation/re-annealing
Fc
eT
Au electrode
Fc
e
T

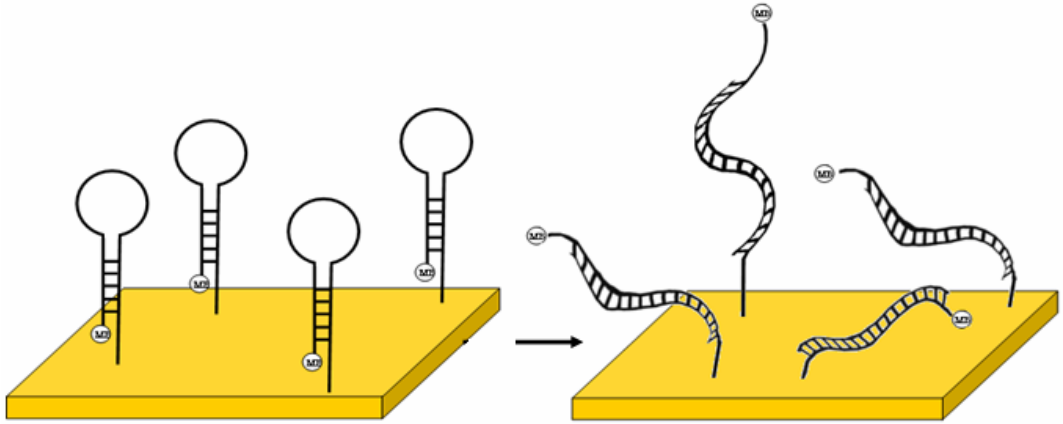

Current (nA)
45
30
15
0
0.3
0.4
0.5
0.6
0.7
E (V vs. NHE)
No target
30 pM
50 nM
5 μM

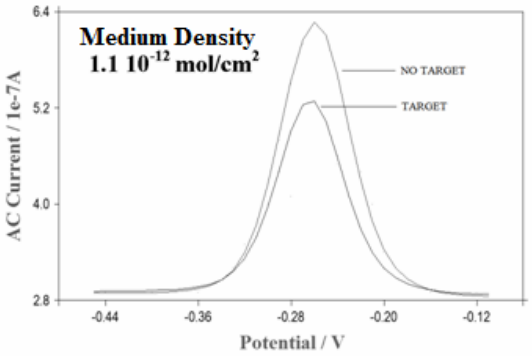
Signaling Mechanism

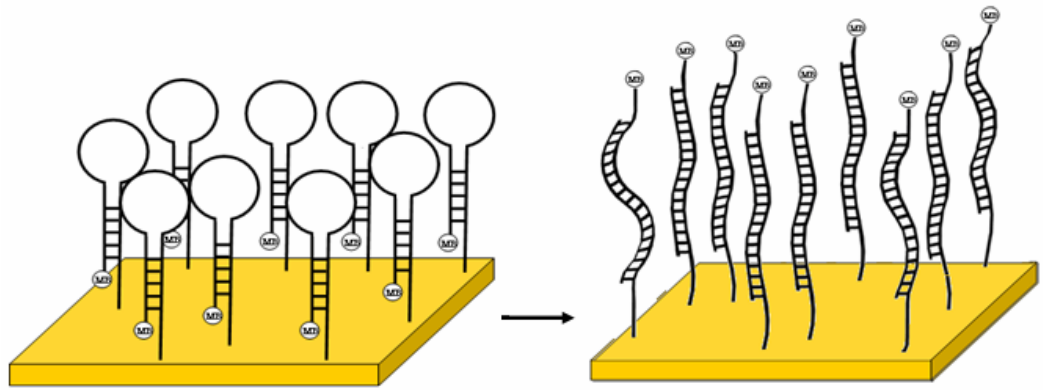


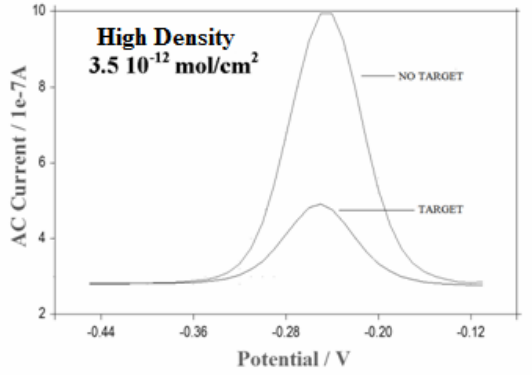
Signaling Mechanism

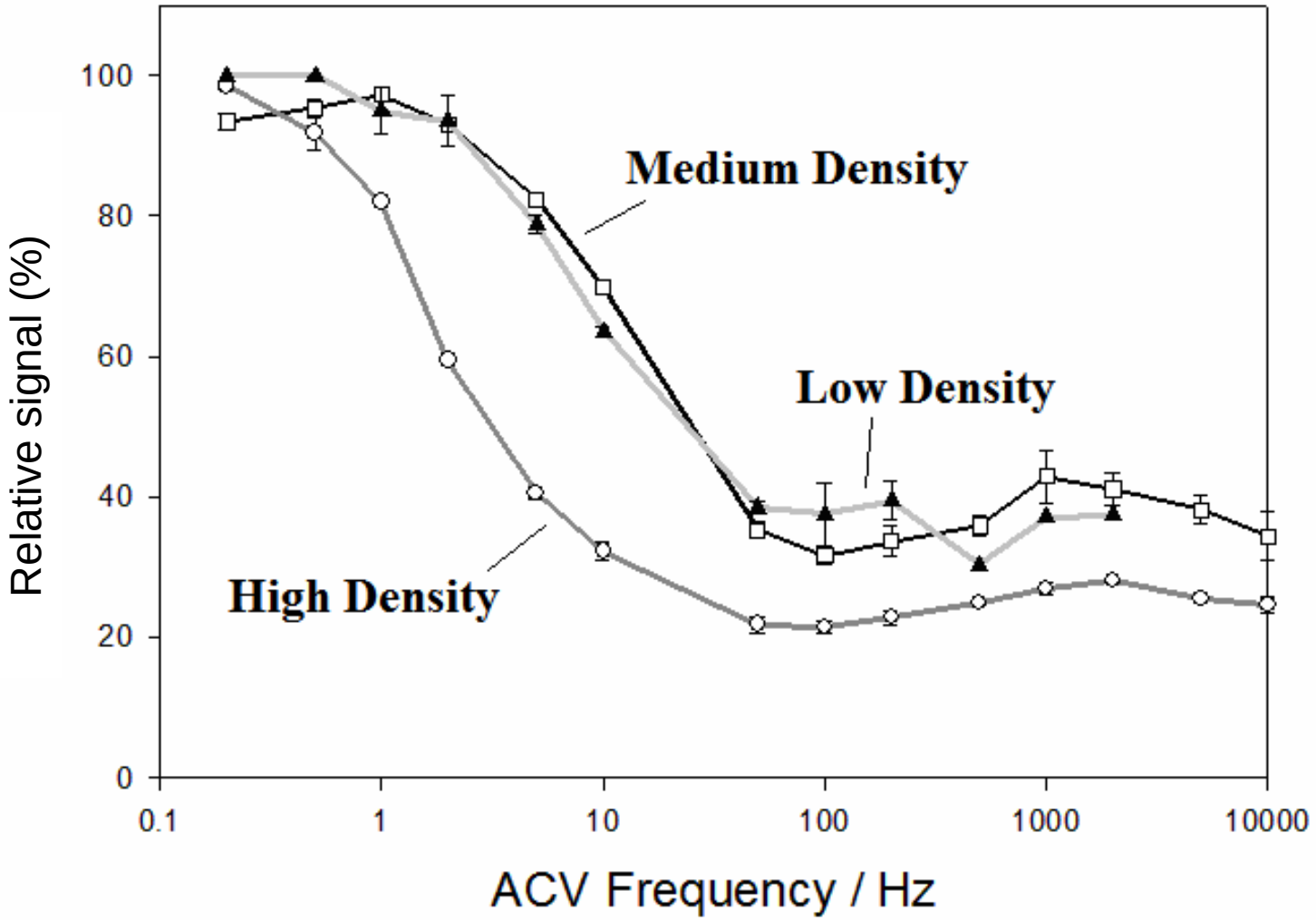




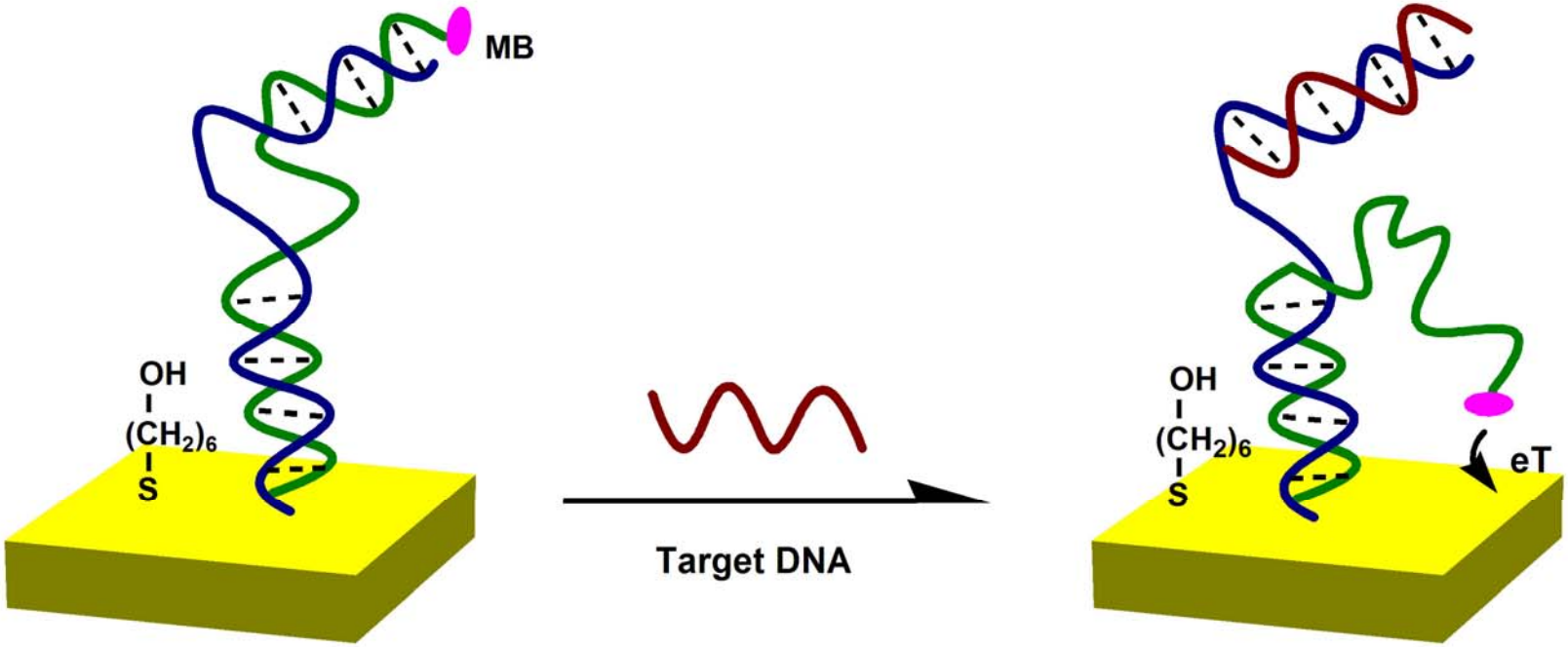

Medium Density
1.1 10-12 mol/cm2
NO TARGET
TARGET
AC Current / 1e-7A
6.4
5.2
4.0
2.8
-0.44
-0.36
-0.28
-0.20
-0.12
Potential / V



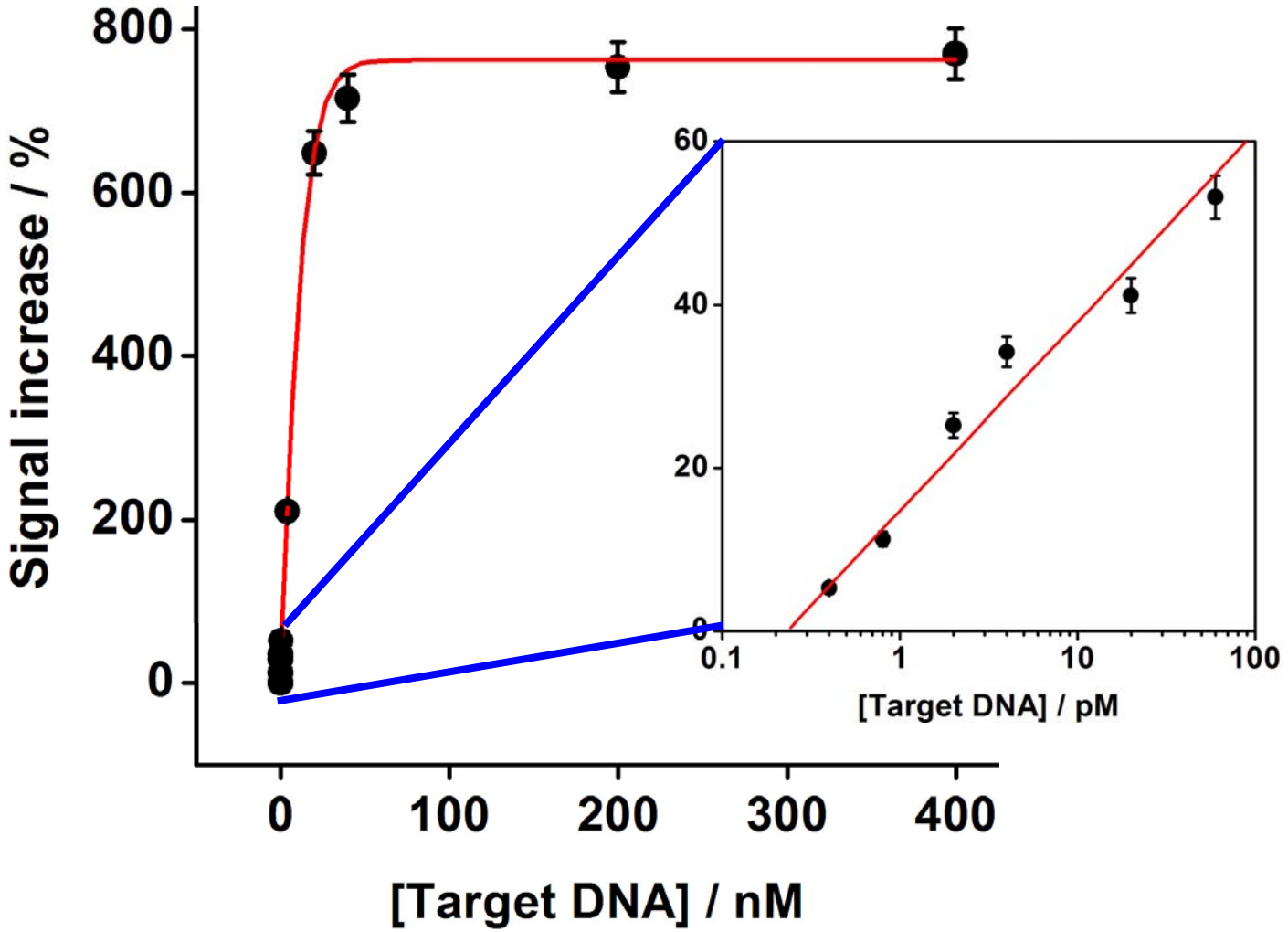

High Density
3.5 10-12 mol/cm2
NO TARGET
TARGET
AC Current / 1e-7A
10
8
6
4
2
-0.44
-0.36
-0.28
-0.20
-0.12
Potential / V

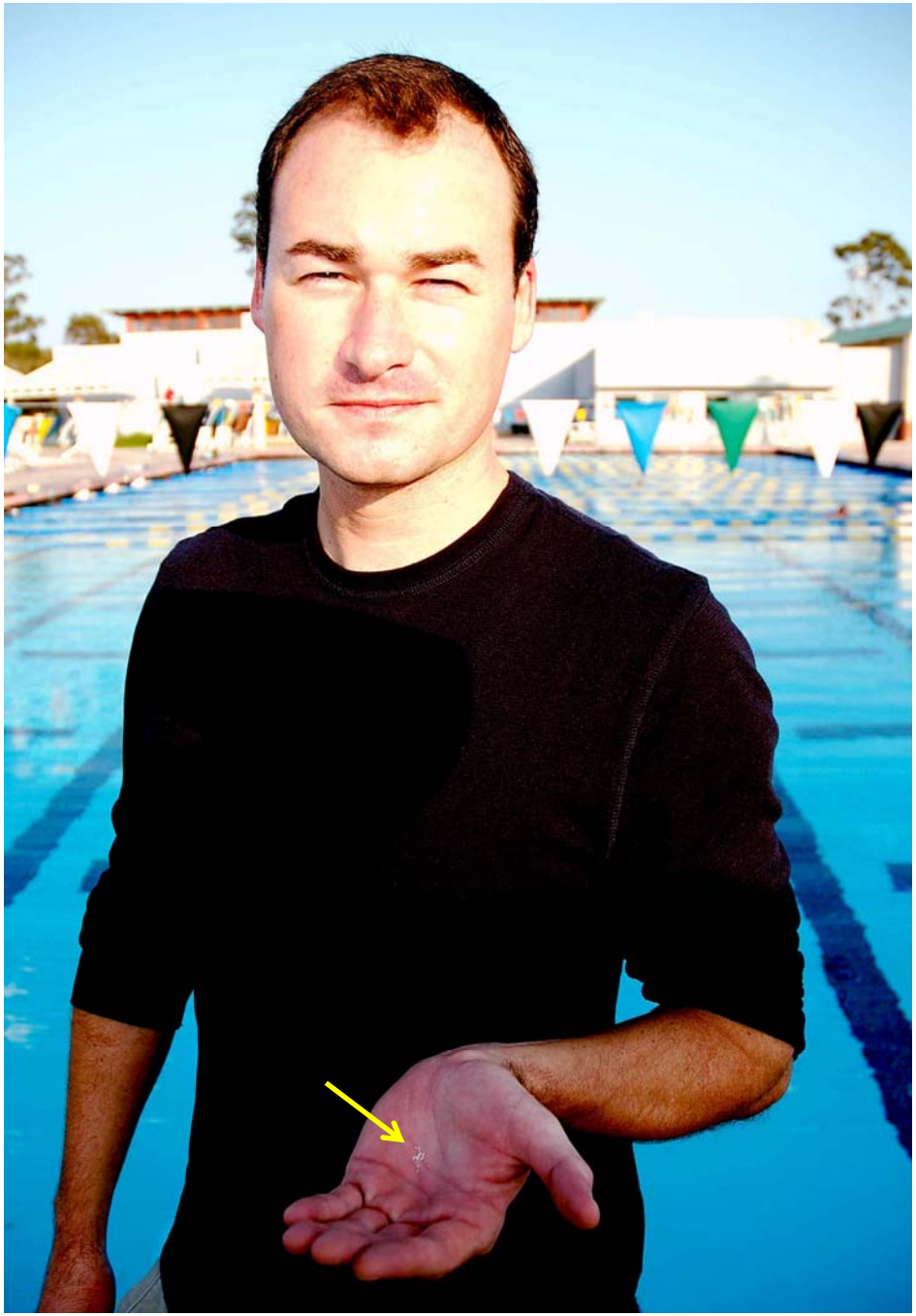

Relative signal (%)
100
80
60
40
20
0
0.1
1
10
100
1000
10000
ACV Frequency / Hz
Medium Density
Low Density
High Density

Signal-On E-DNA



Femtomolar Detection



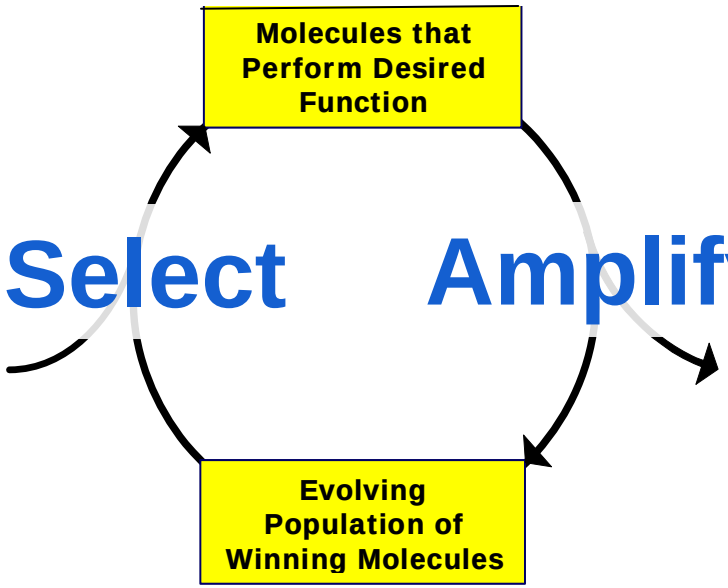


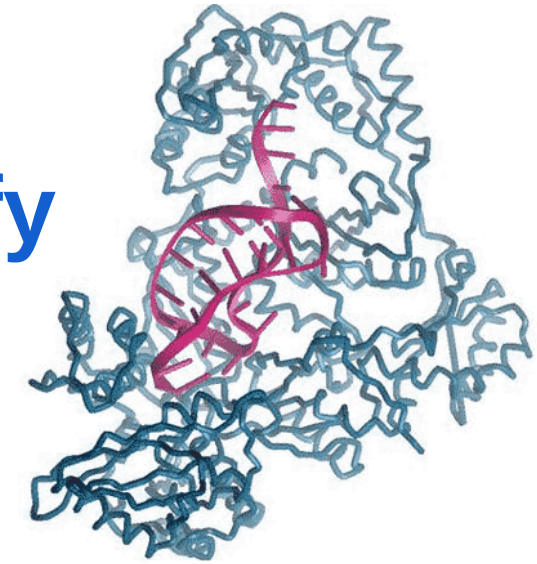
100 fM

$$\frac{\text{1.5 mg DNA}}{\text{2.5 x } 10^6 \text{ L}} = \text{600 ppq}$$

Aptamers

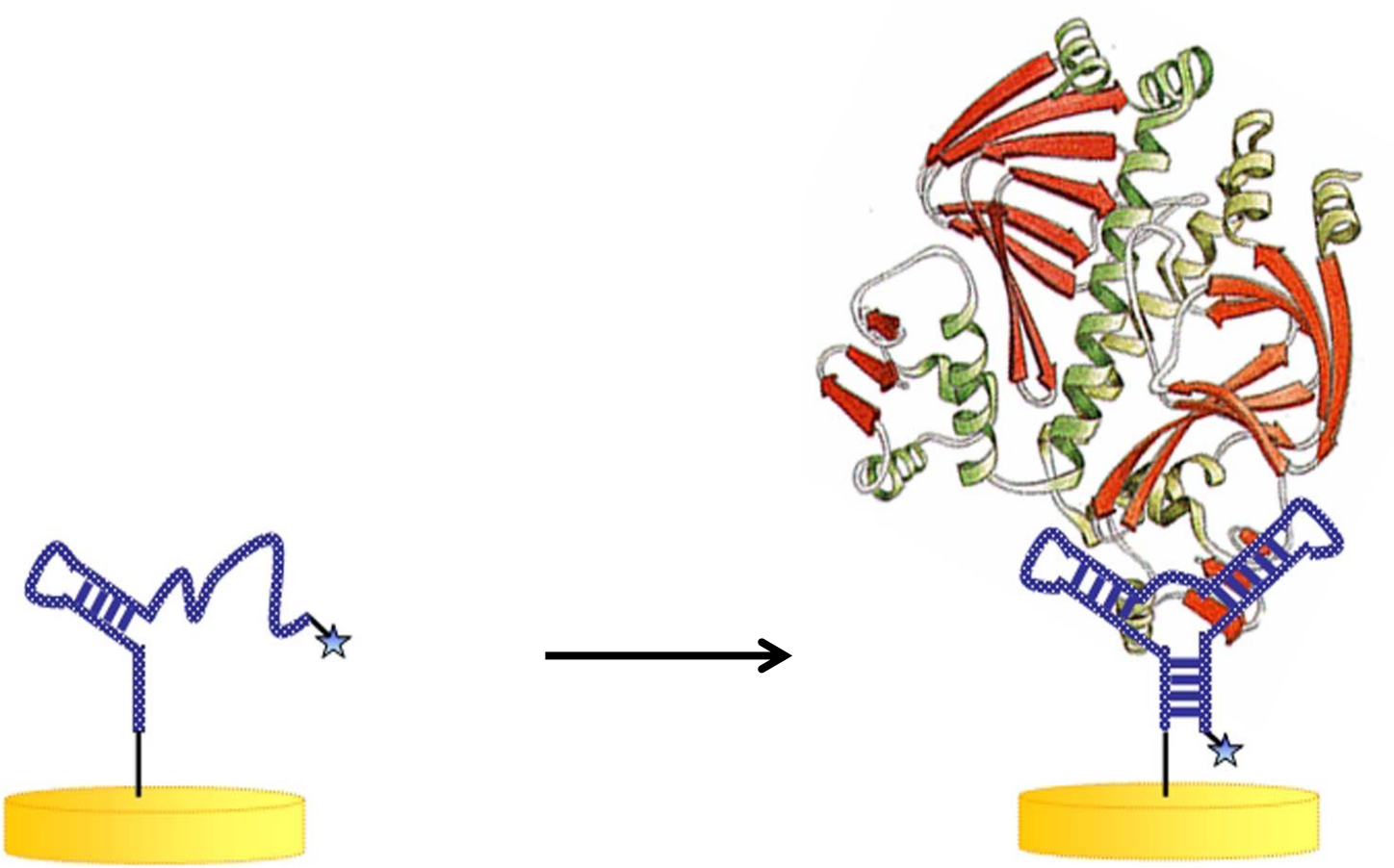
Random library of 10^{14} unique DNA sequences



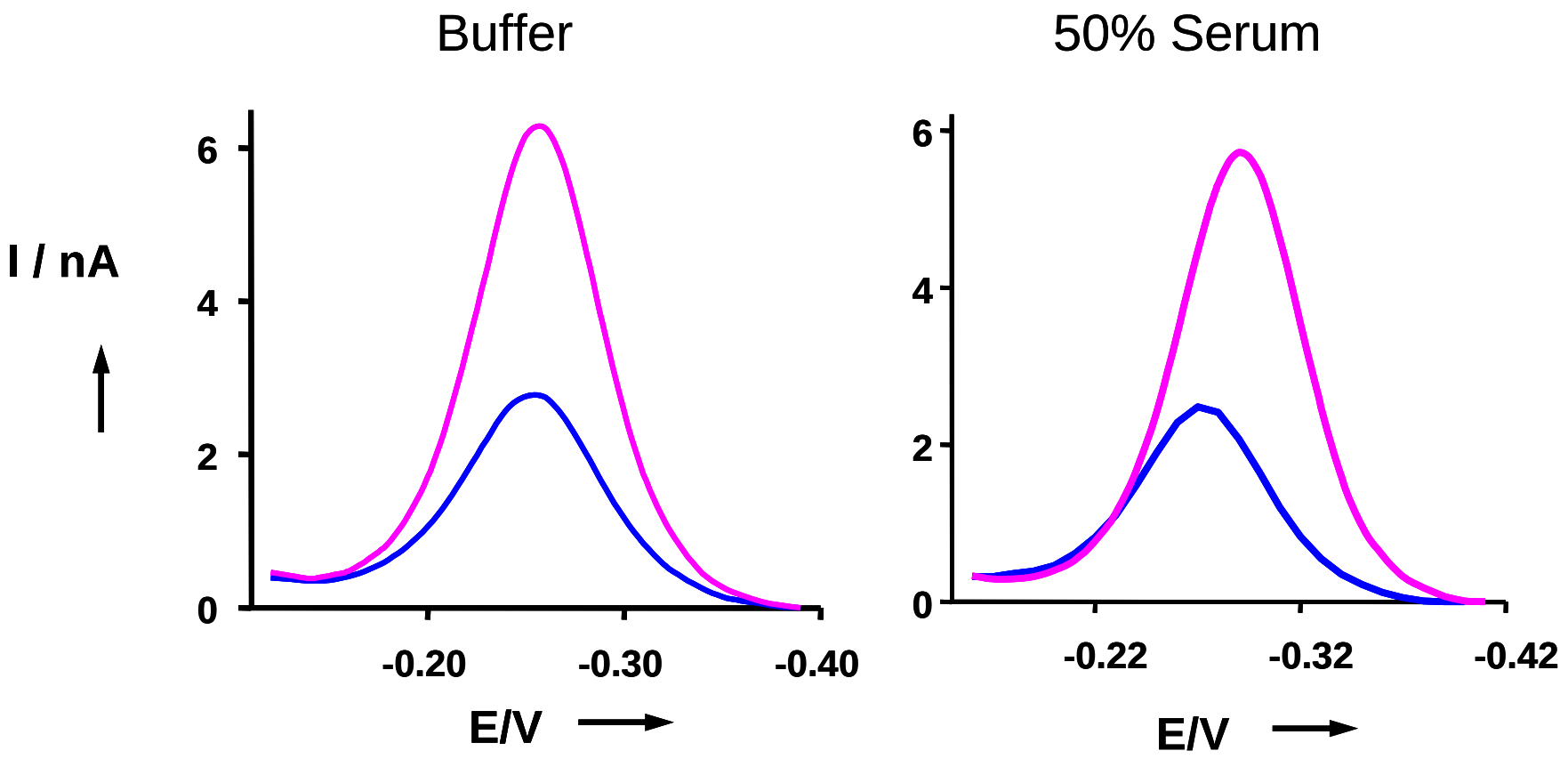


High Affinity Aptamer

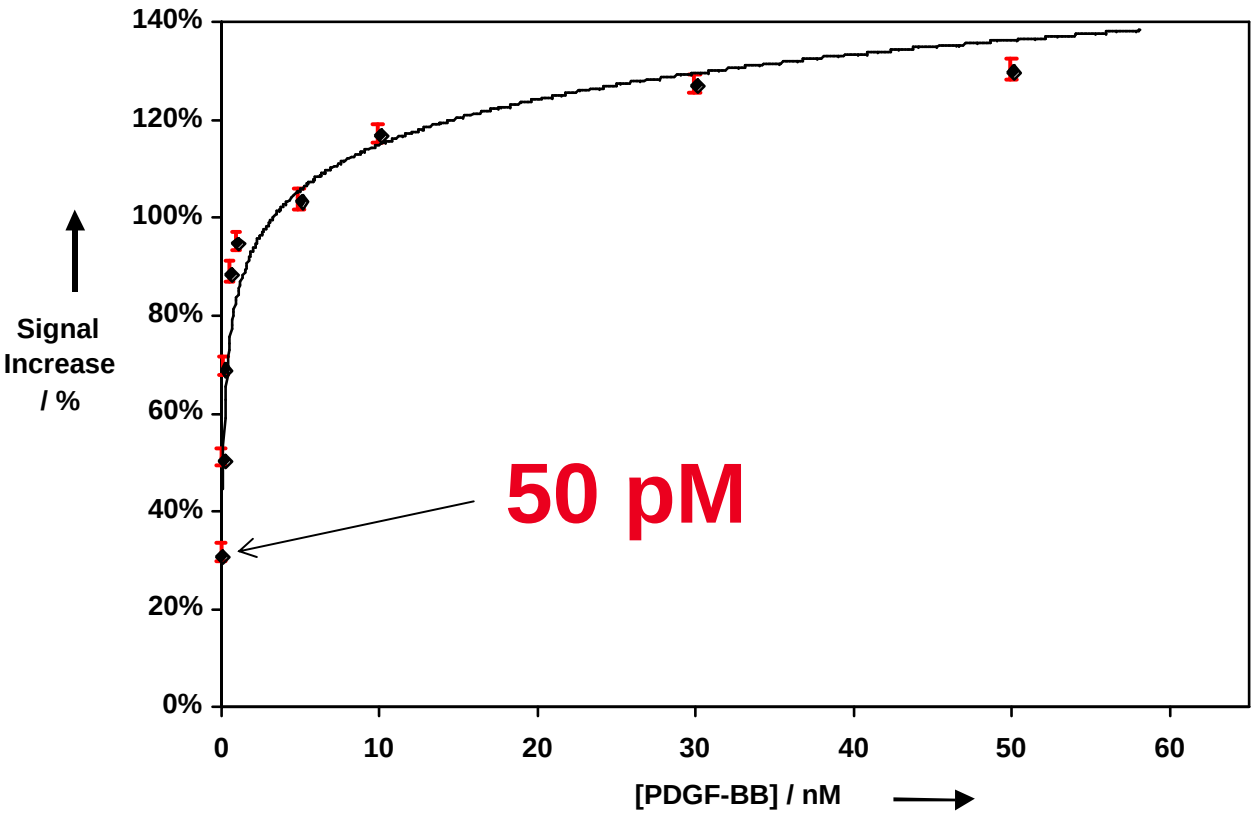
E-AB Sensors



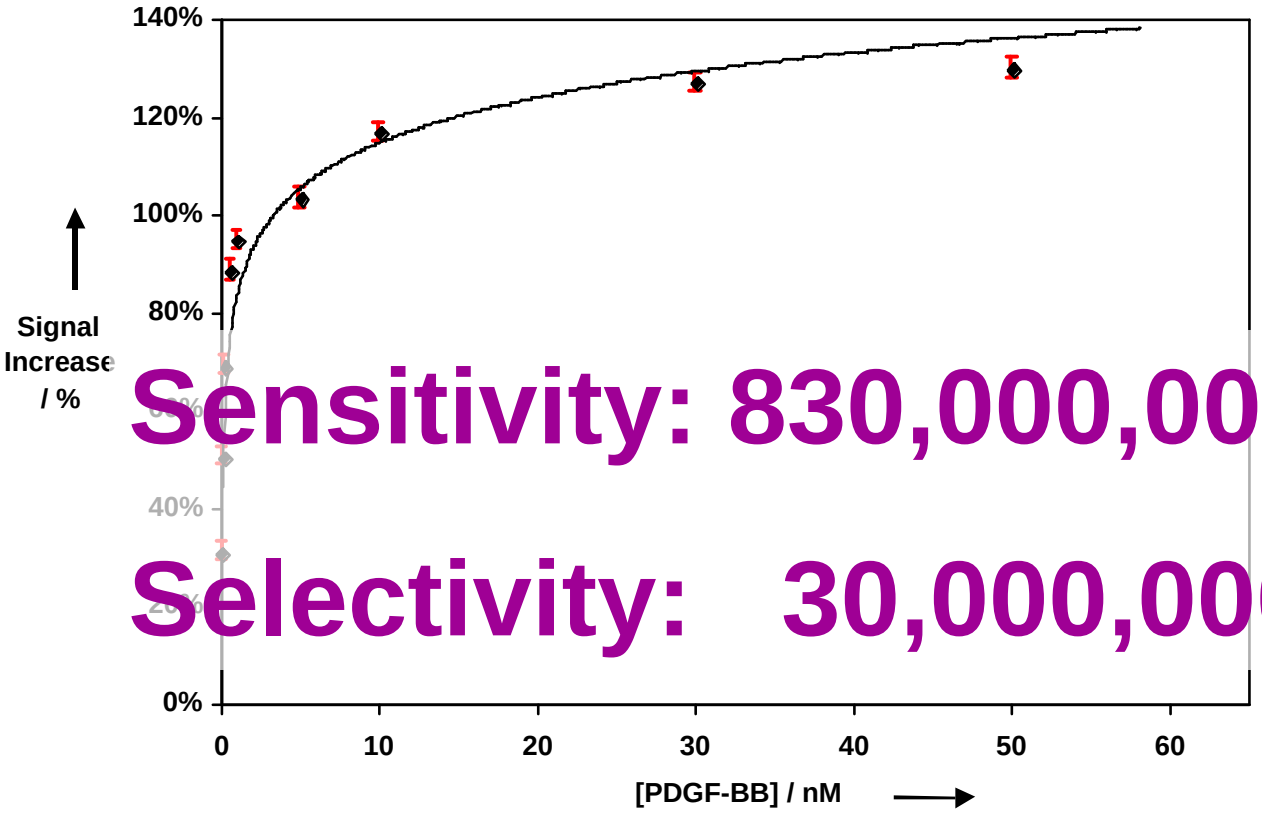
PDGF Detection in Blood



PDGF Detection in Blood



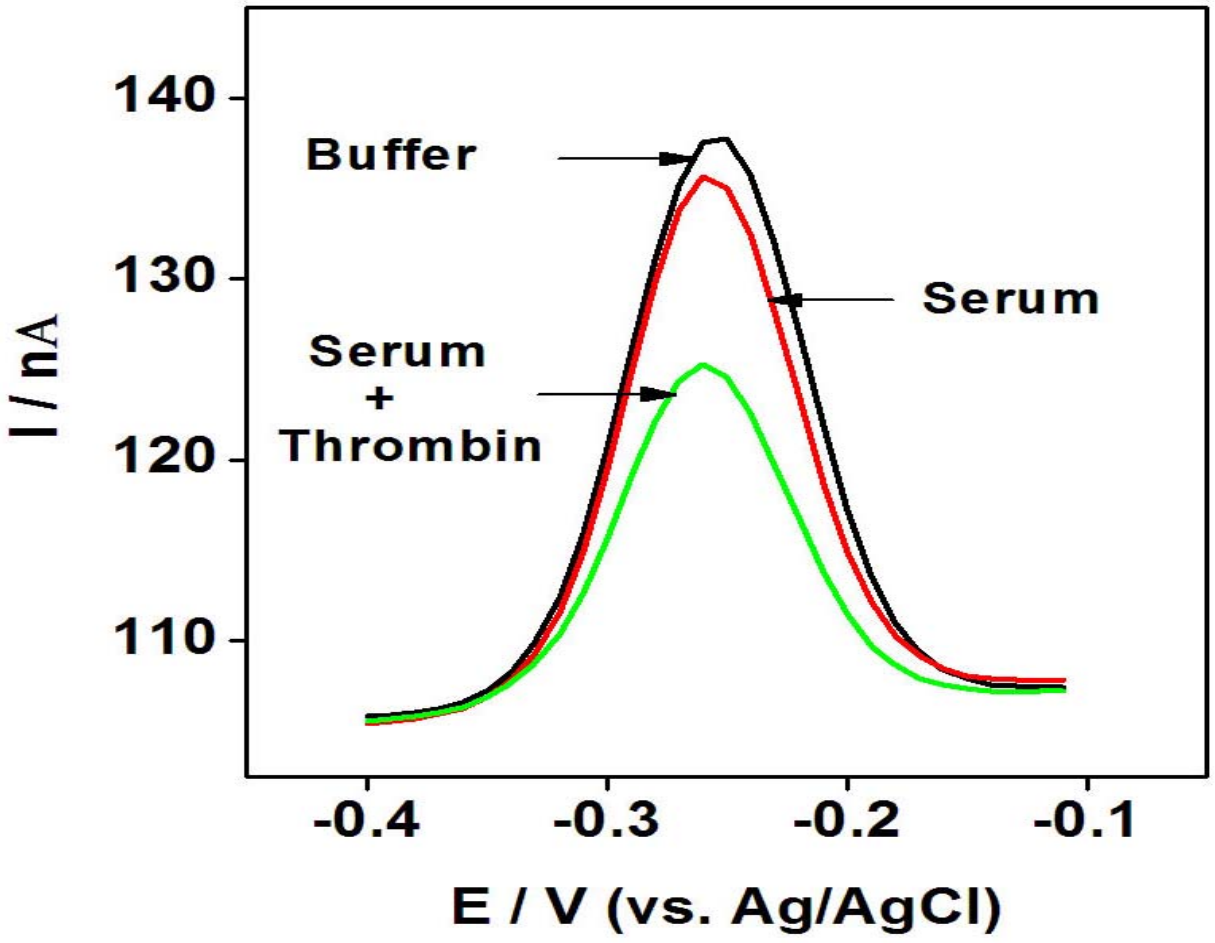
PDGF Detection in Blood



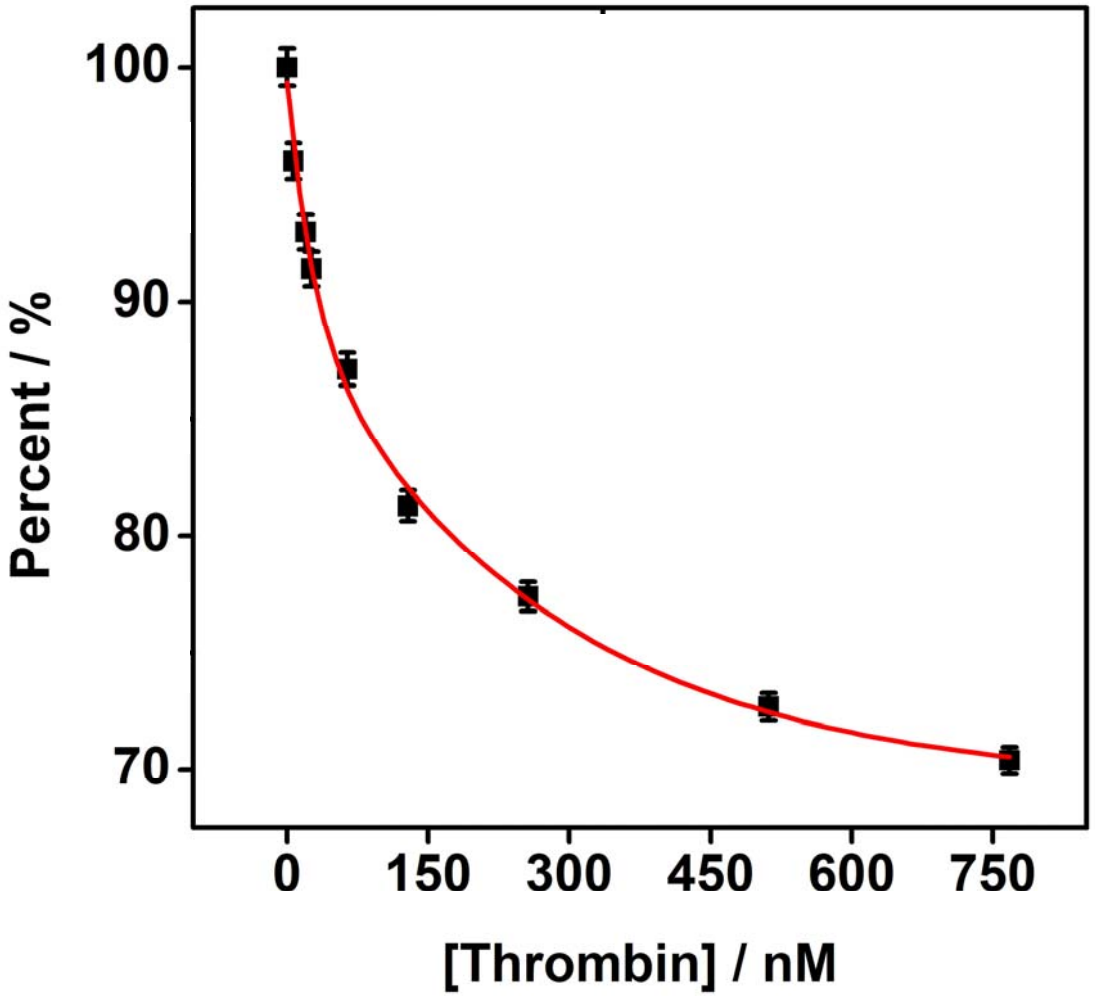
Sensitivity: 830,000,000:1

Selectivity: 30,000,000:1

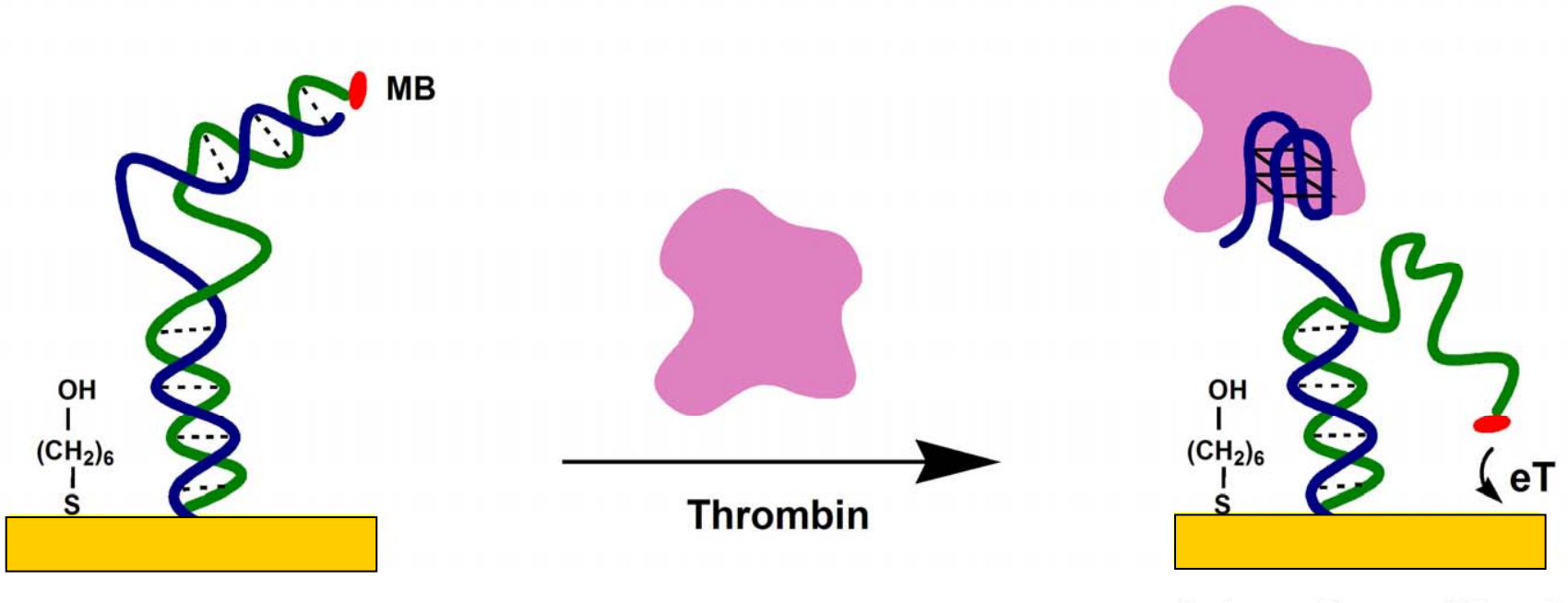
Thrombin Detection



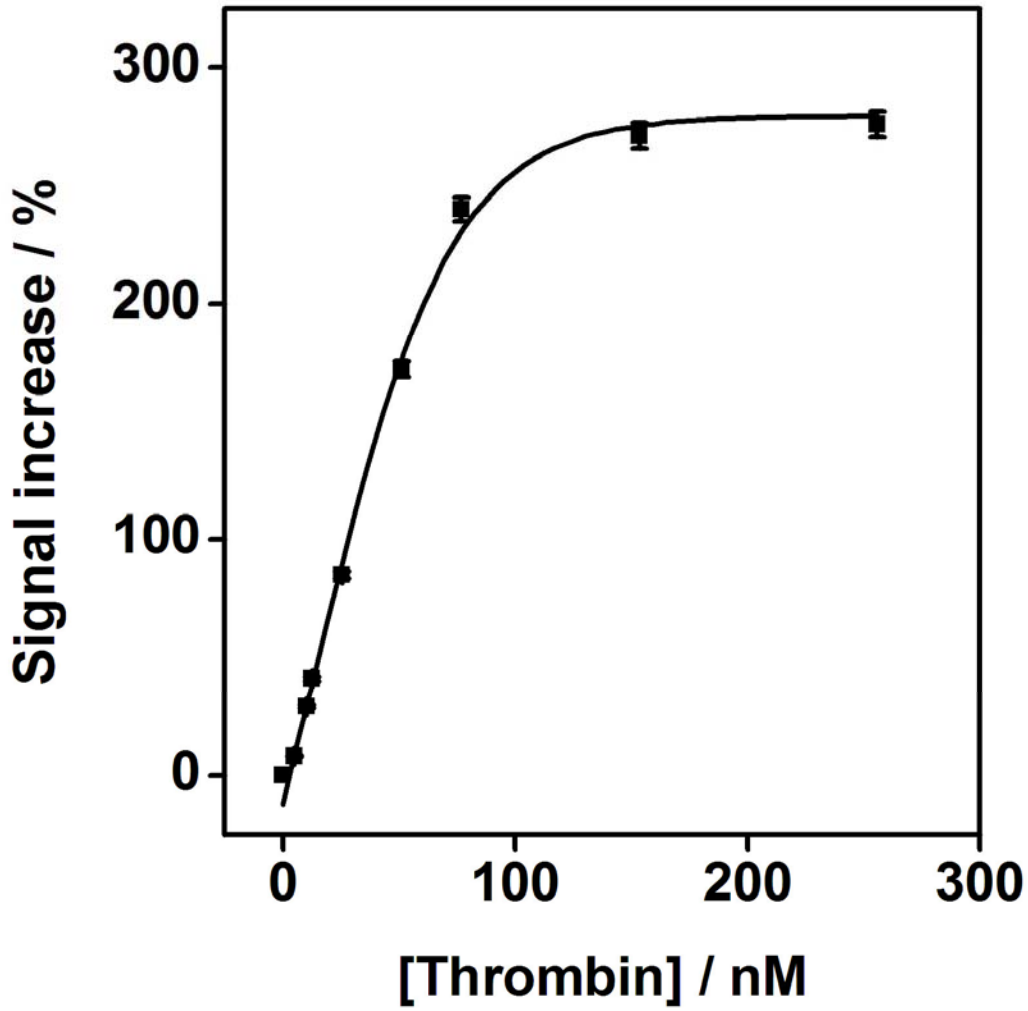
At Physiological Concentrations



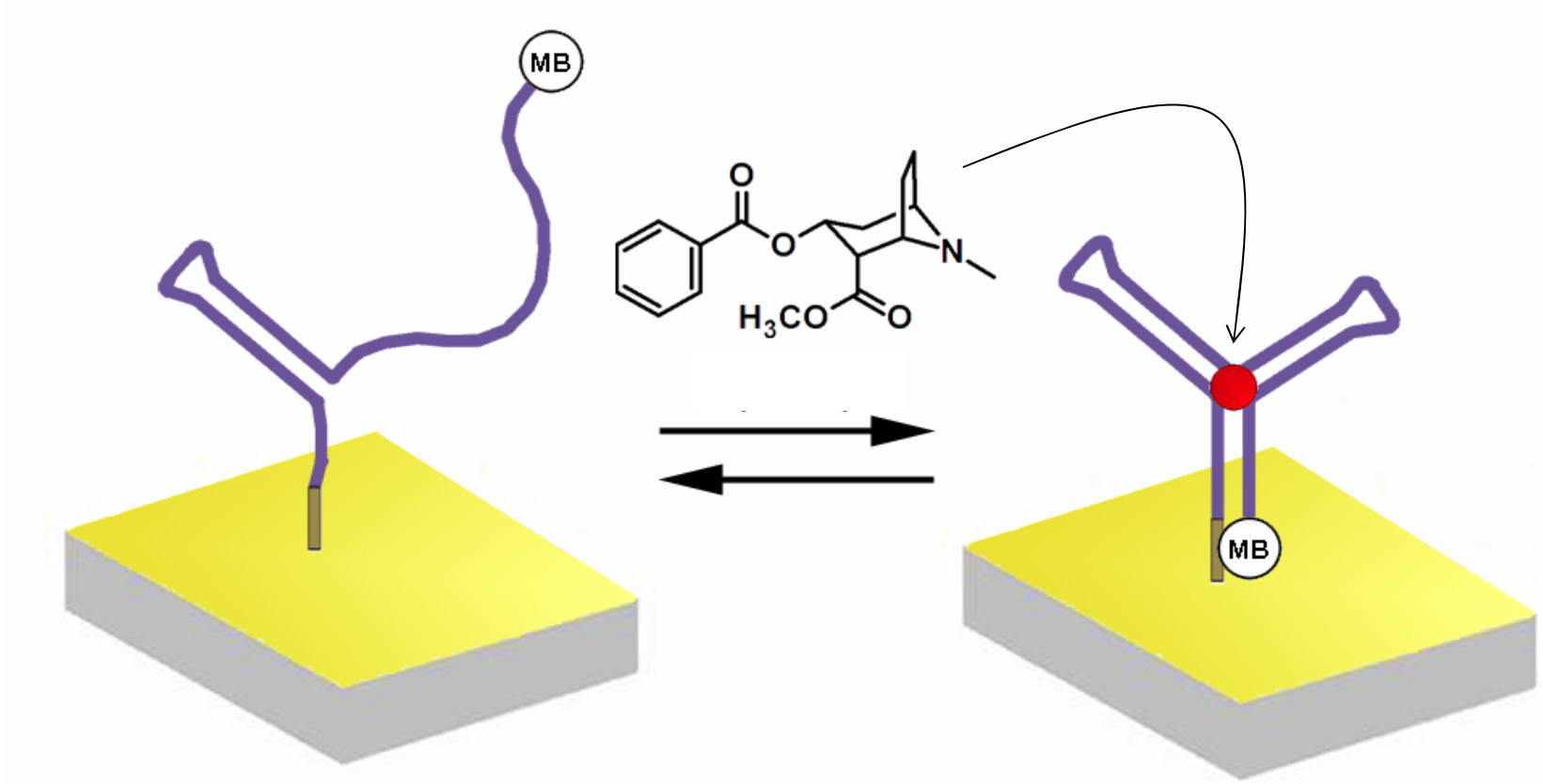
Signal-on E-AB Sensor



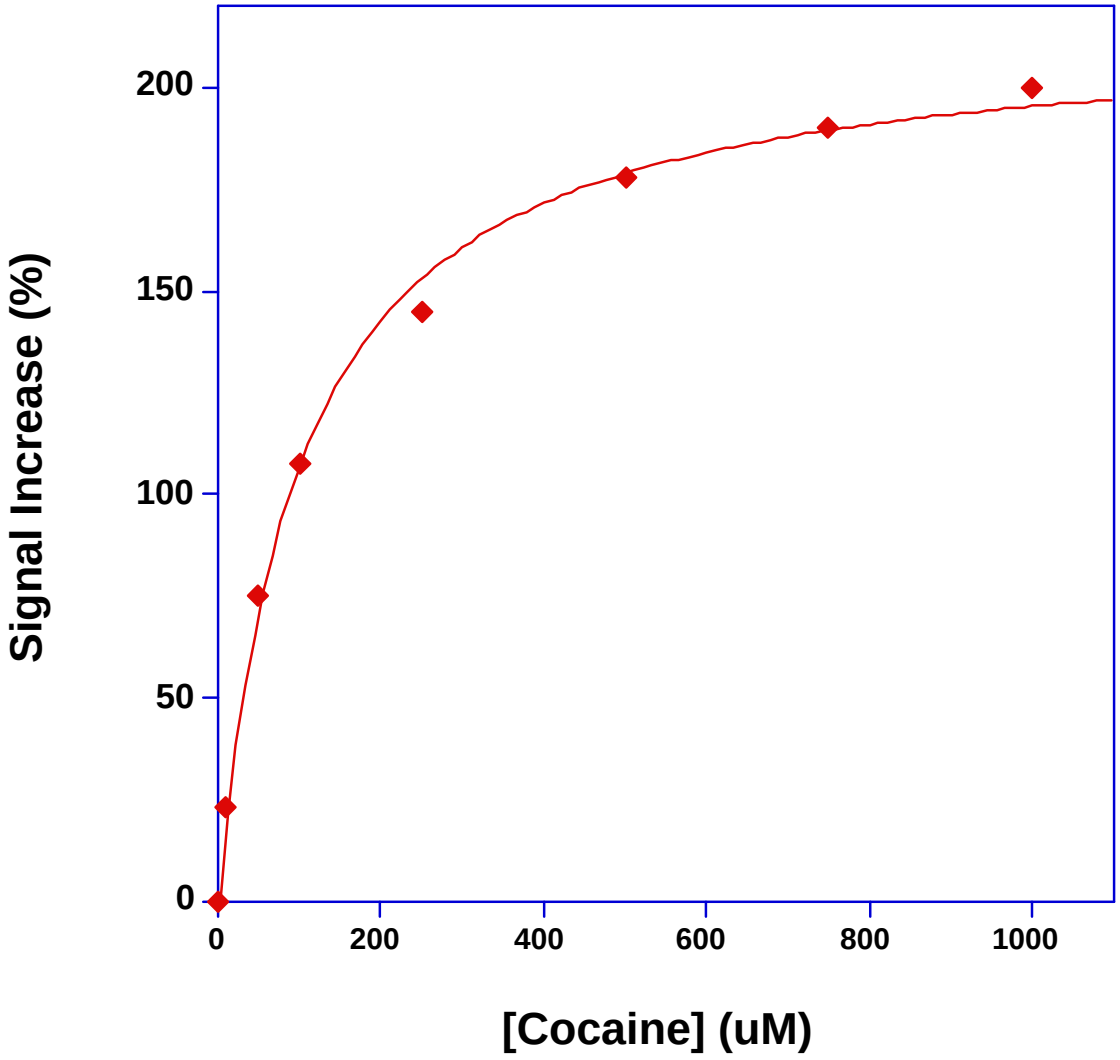
Signal-on E-AB Sensor



Small Molecule Detection



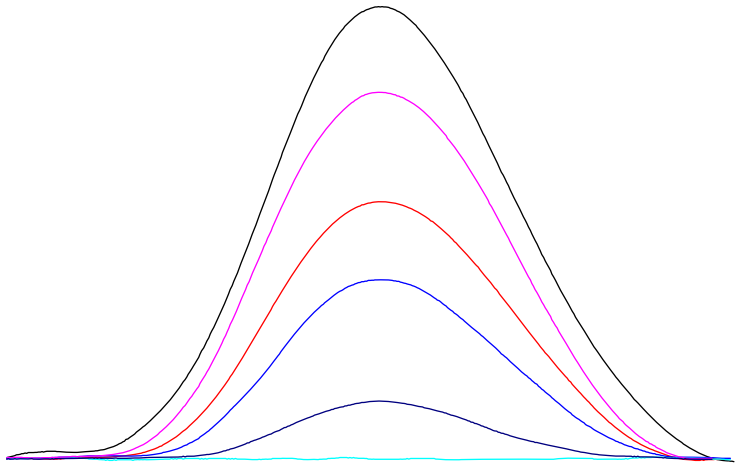
Cocaine in Blood Serum

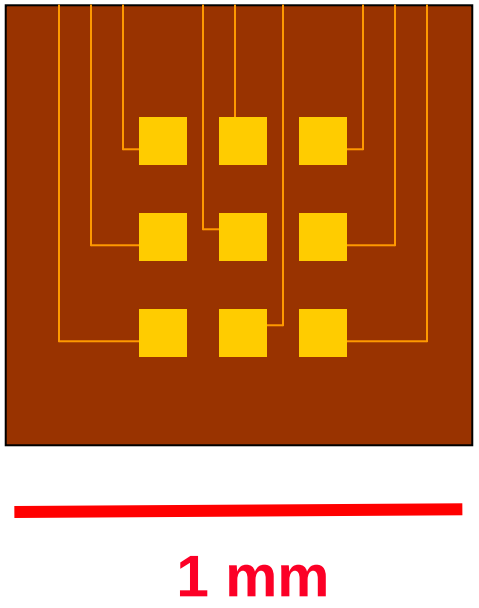



1 μM = ~1 kg / 2.5 x 10⁶ L = 400 ppb
End

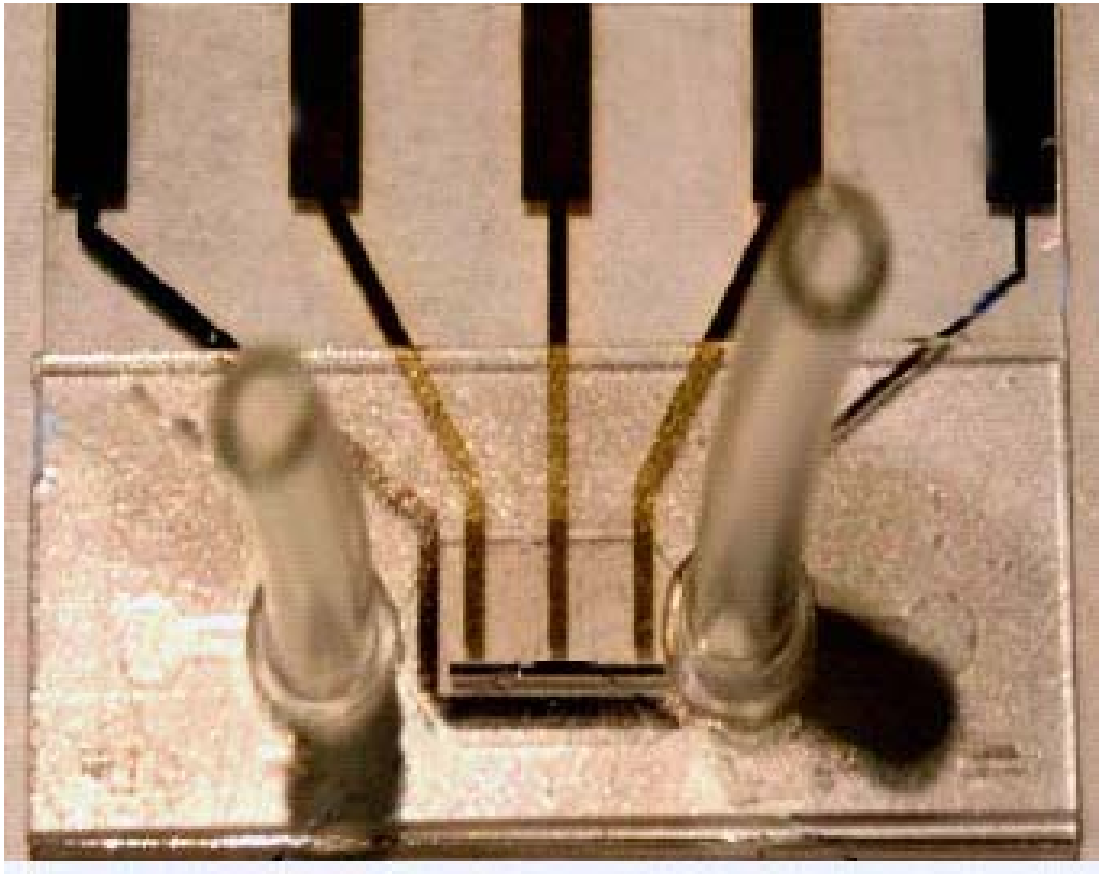
Device Integration

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.





Monolithic Microfluidic Device



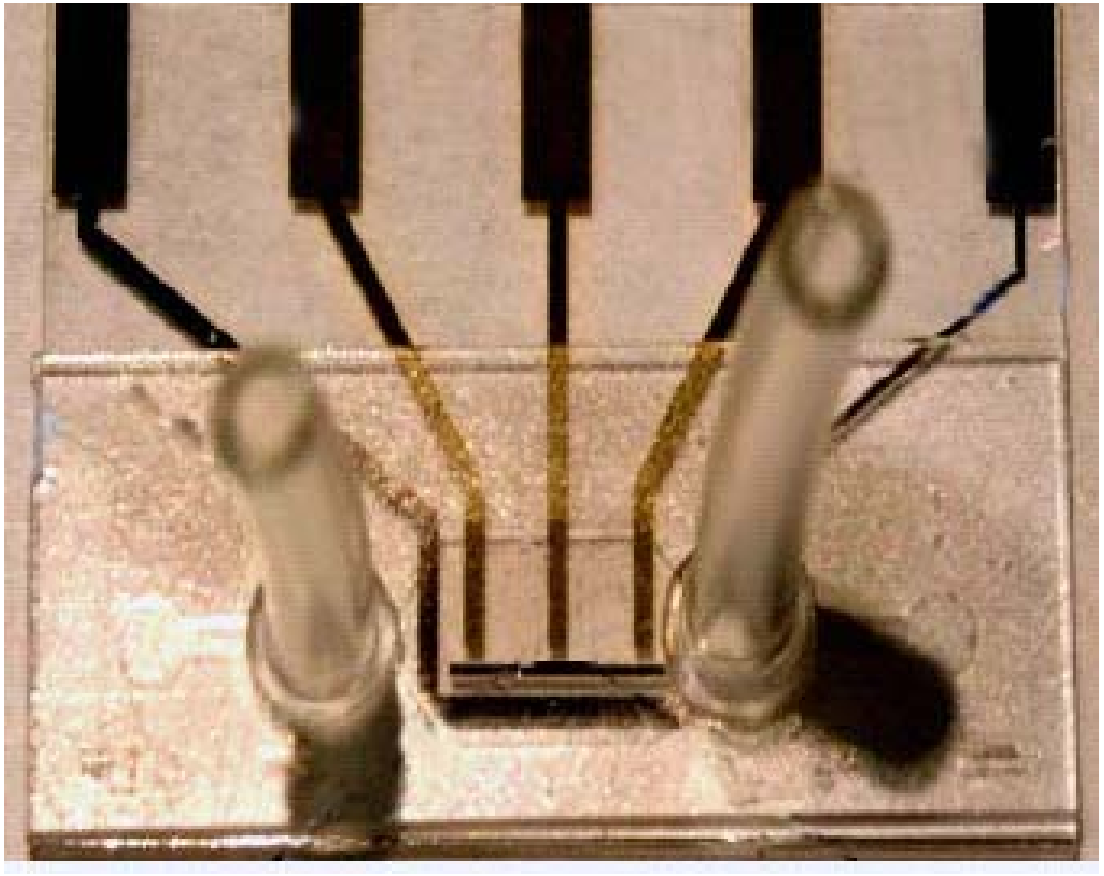
Monolithic Microfluidic Device

All glass substrates

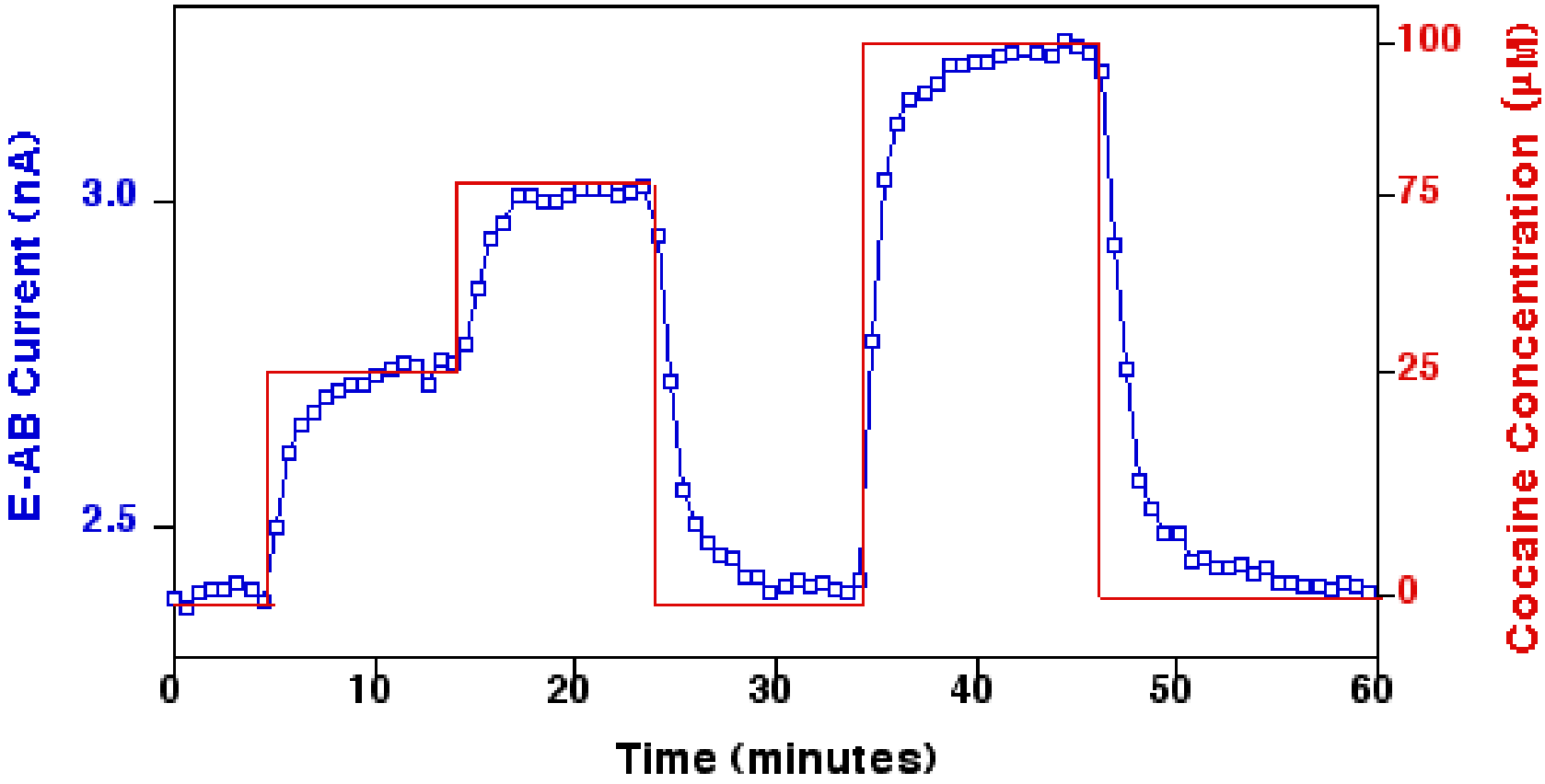
Glass-on-glass bonding

Improved SiN insulation

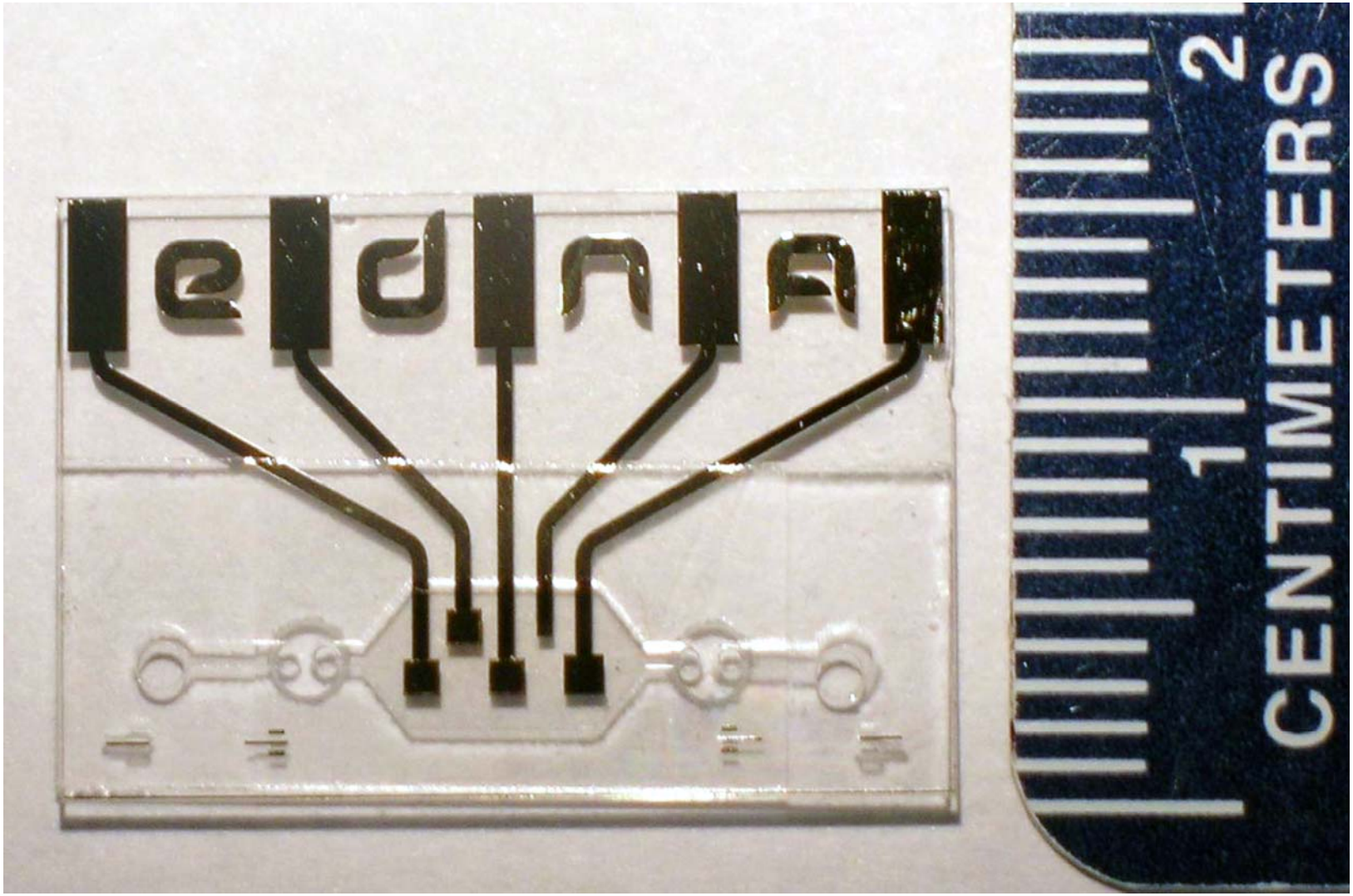
Low-acid cleaning



Real-time Cocaine Detection in Flowing Blood Serum



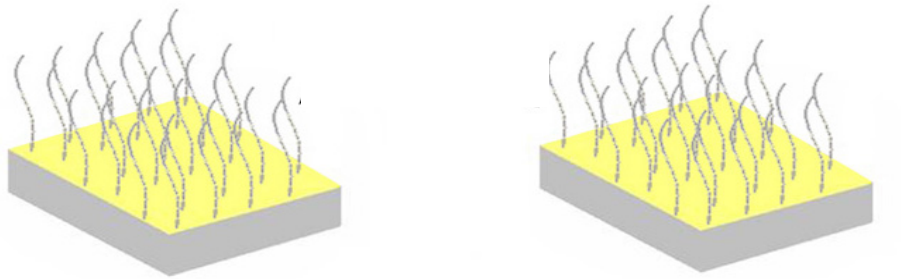
Multi-analyte Devices



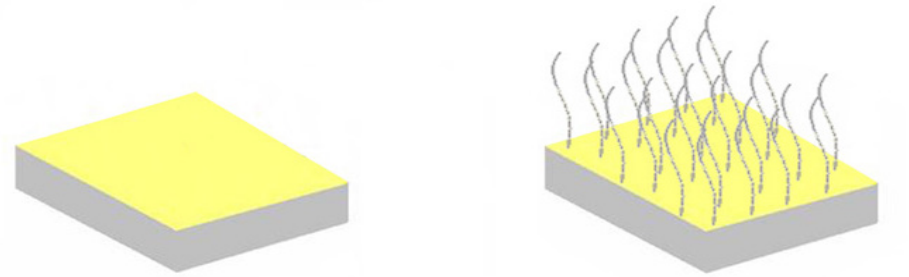
Electrochemical Lithography

Alkane Thiol

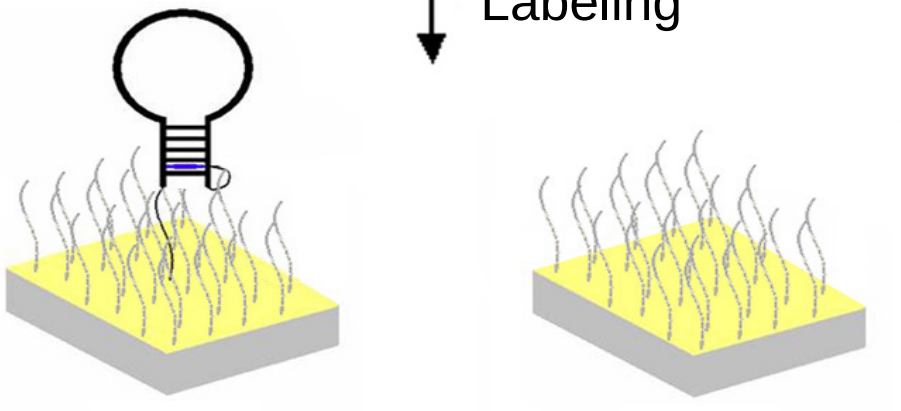
Passivation



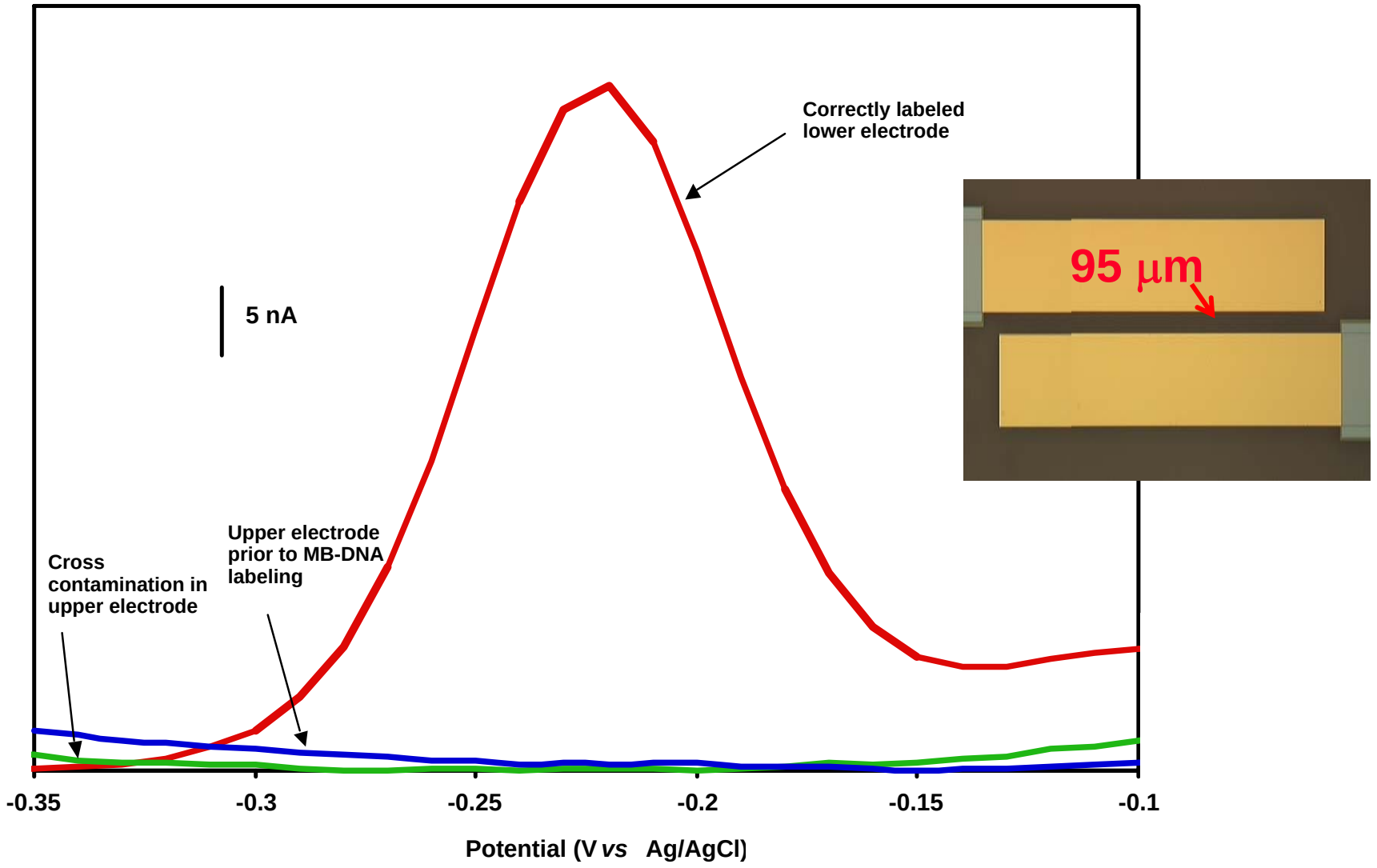
Oxidative Stripping



Labeling



Differential Labeling

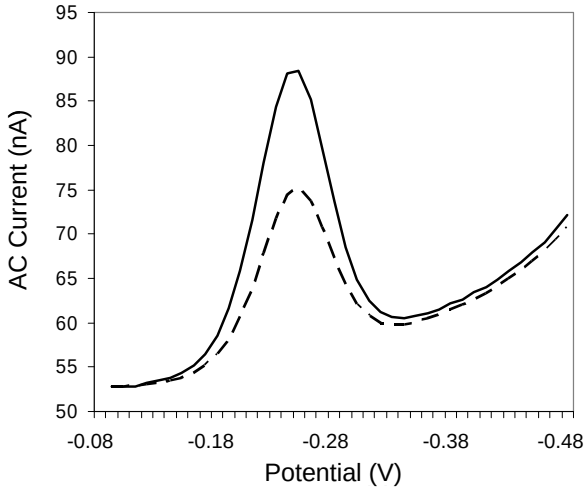


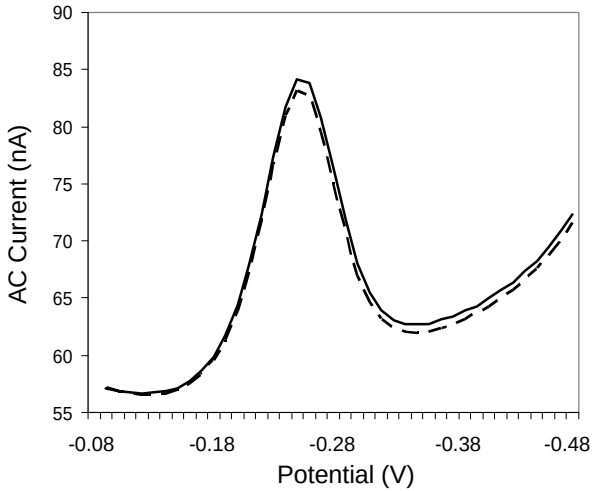
Two-Pixel Device

H1N5 Probe

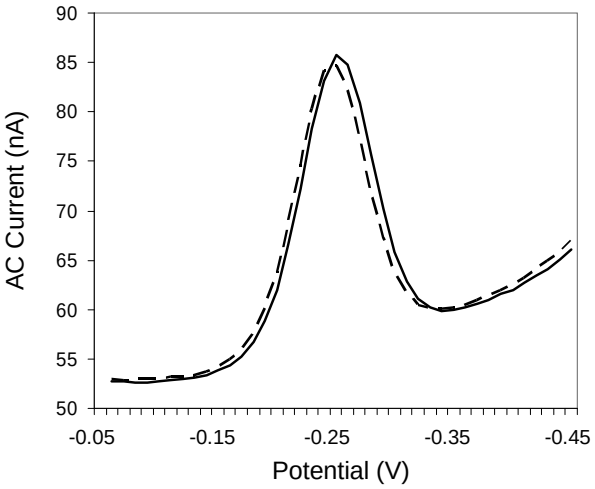
H1N1 Probe

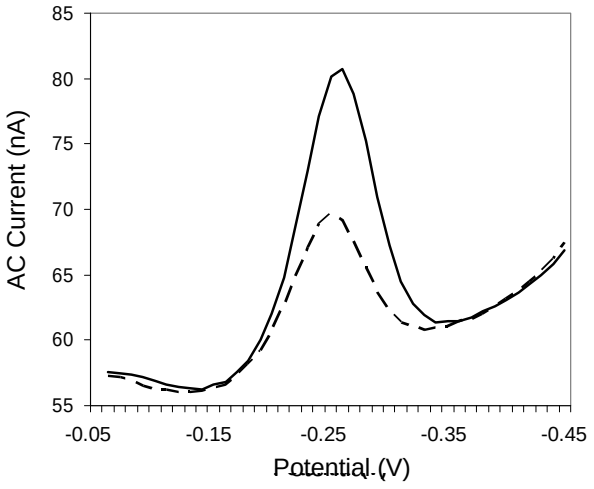
Avian Flu Target



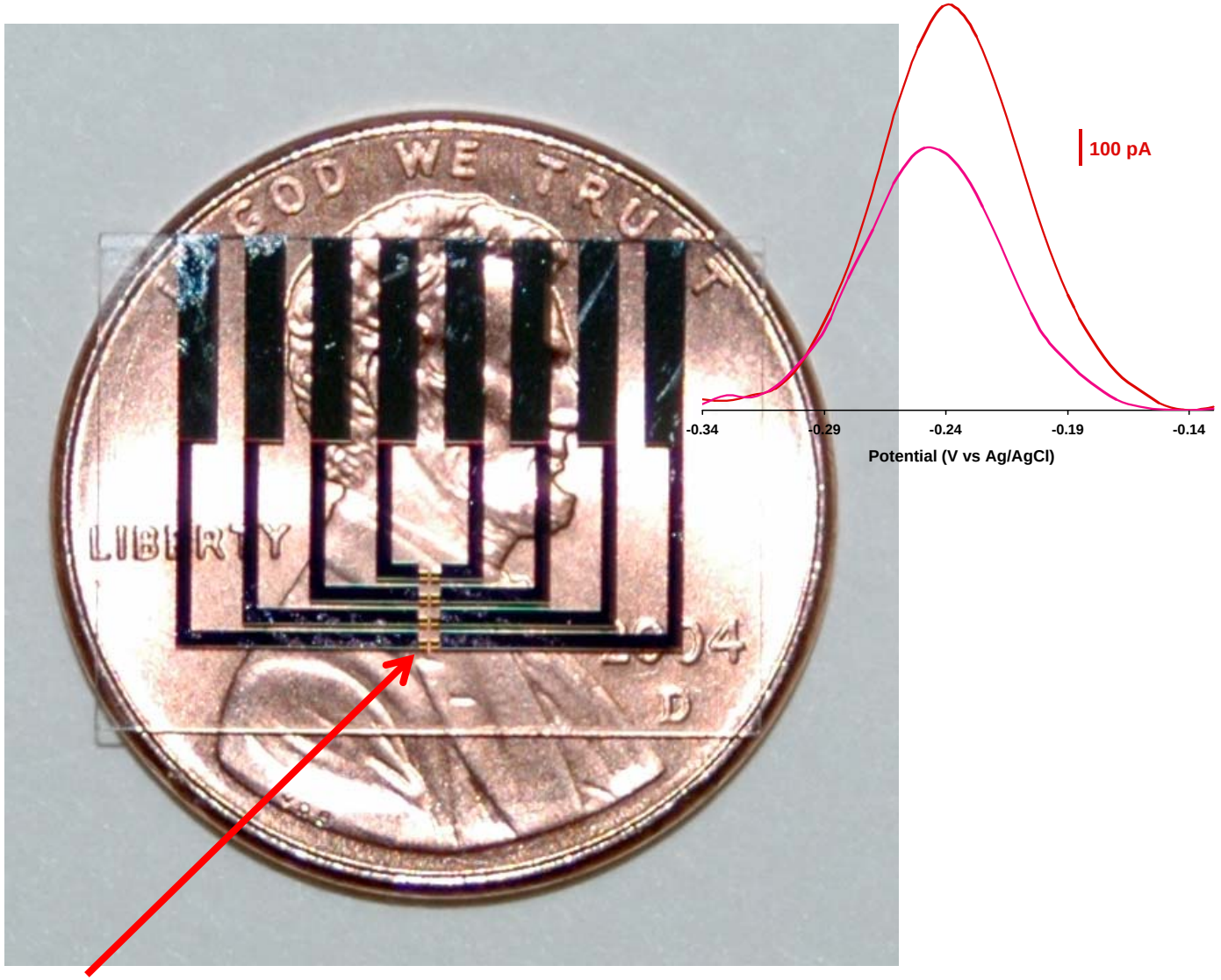


Human Flu Target



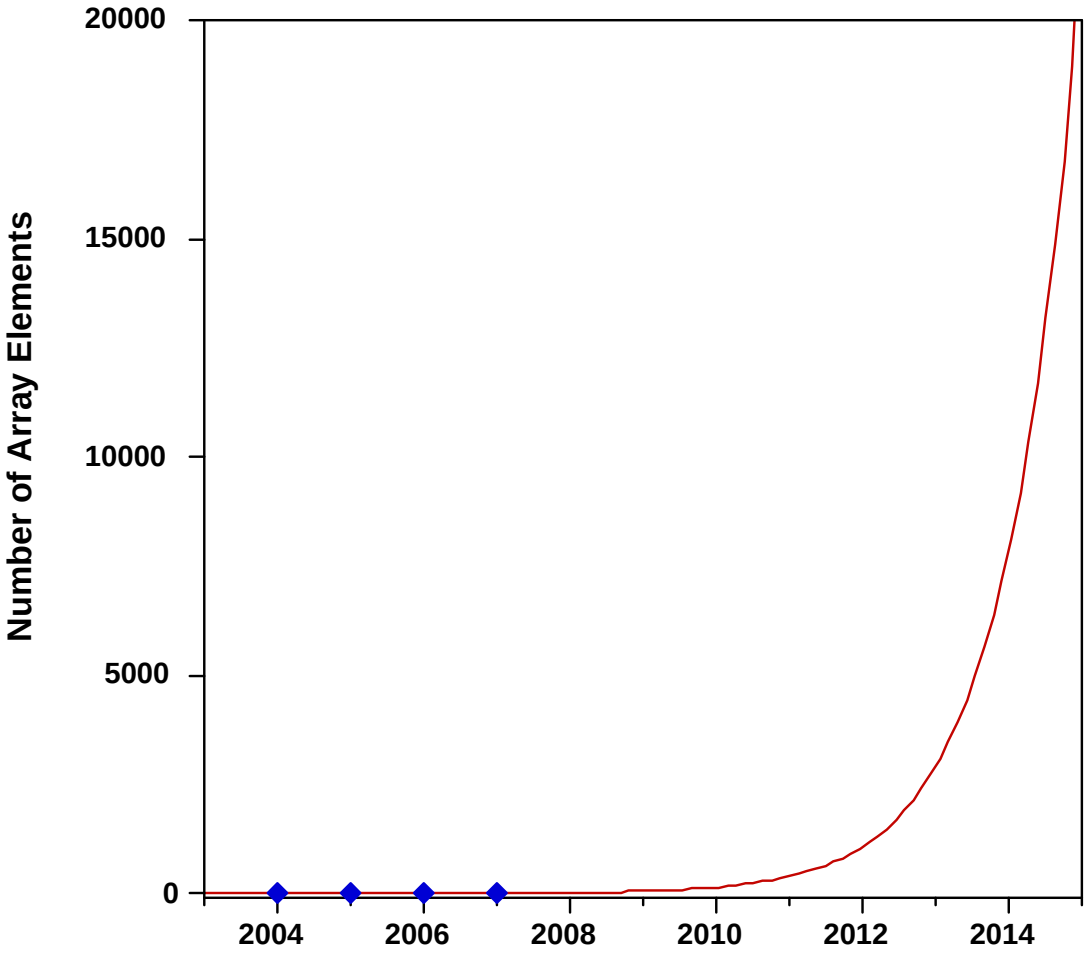


Multi-Analyte Arrays



8 pixels @ 24 pixels/mm^2

Moore’s Law


Number of Array Elements
20000
15000
10000
5000
0
2004
2006
2008
2010
2012
2014

Jonathan Kohn

Ken Oh

Kevin Cash

ChunHai Fan

Arica Lubin

Yi Xiao

Rebecca Lai

Brian Baker

Alan Heeger

Jim Sumner

Tom Soh

Eric Lagally

James Swenson

Sang-Ho Lee

Liz Pavlovich

NIH, ONR, DARPA (CNID), ARO (ICB), UARP,
Clinical Microsensors, Inc., DOE (LLNL)